A STUDY OF CORDBLOOD CHOLESTEROL & TRIGLYCERIDES IN NEW BORN BABIES

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C E R T I F I C A T E

This is to certify that the work entitled "A STUDY OF CORD BLOOD CHOLESTEROL AND TRIGLYCERIDES IN NEW BORN BABIES", which is being submitted as a thesis for M.D.(Paediatrics) examination, 1991 of Bundelkhand University, has been carried out by Dr. RAGHVENDRA NATH DWIVEDI in the department of Paediatrics, M.L.B. Medical College, Jhansi.

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(Raghvendra Nath Dwivedi)

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INTRODUCTION

INTRODUCTION

The rapidly sweeping pandemic of ischaemic heart diseases has lead to a search for newer strategies to prevent atherosclerosis in its early stages. Because of a greater risk of developing premature and accelerated coronary atherosclerosis early detection of children with familial hyperlipoproteinemia with subsequent dietary intervention seems to be an attractive approach. Neonatal familial type II hyperlipoproteinemia may often be identified by elevated levels of cord blood cholesterol and triglycerides (Glueck et al, 1971).

The cord blood lipid levels are much less likely to be influenced by extraneous factors as compared to that in any other period of life. A general dissimilarity between the cord blood and maternal cholesterol and triglycerides levels has been described at the time of parturitian suggesting that maternal lipids and lipoproteins do not cross the placental barrier (Kaplan and Lee, 1965). However, some evidences have been gathered to suggest that ante partum factors such as maternal hypertension, antepartum haemorrhage, foetal anoxia or intrapartum compromise such as prolonged labour and leaking P/V meconium stained liqour amnii and post maturity may be associated with hyperlipidemia (Tsang et al, 1974; Cress et al, 1977).

It has been suggested that hyperlipoproteinemia can be diagnosed at birth by elevated levels of umbilical cord cholesterol, although opinions to the contrary have been offered and babies with elevated cholesterol at birth had values distributed through normal range when re-examined at one year age (Dermandy et al, 1972).

In small for date babies with intrauterine malnutrition - which favours adipose tissue breakdown liberating free fatty acids, the portion of free fatty acids which escapes oxidation for energy is converted in the liver into triglyceride, resulting into rise in blood triglyceride levels. Full term babies on the contrary are in receipt of ready placental supply of nutrients so there is little need of lipolysis in utero (Haridas et al, 1984). Preterm delivery is not a normal physiological phenomenon and it involves some amount of stress to fetus which may or may not be manifest clinically (Kumar et al, 1989). Stress in any form has been shown to raise serum triglyceride levels (Cress and Shaher, 1977).

The assessment for hyperlipidemia can be done by serum cholesterol, serum triglyceride, serum lipoprotein and serum free fatty acid assays. Lipoprotein electrophoresis is not useful as a screening tool as it is only semiquantitative. A complete quantitation of lipoprotein by ultra-centrifugation is not usually accessible and is quite expensive (Kwiterovich, 1982).

Low density lipoprotein cholesterol estimation though more sensitive is only possible at highly specialised centres. Therefore, simple cholesterol and triglycerides estimation is still retained as preliminary screening tests, and it is possible that quantitation of cord blood cholesterol and triglycerides might provide a rapid, easily available and inexpensive measures that could be used prospectively to forecast hyperlipidemia and atherogenic problems, and retrospectively might provide a means of reviewing maternofetal factors that could have contributed to fetal stress.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Lipids are a heterogenous group of compounds related either actually or potentially to fatty acids. They have the property of being relatively water insoluble and soluble in non polar solvents such as ether, chloroform and benzene.

Lipids are important, because of their high energy value, importance of fat soluble vitamins and fatty acids, and because of thermal insulation provided by them.

Bloor (1969) has classified lipids into simple, compound and derived lipids. Simple lipids are esters of fatty acids with various alcohols. They include fats (cholesterol, triglycerides) and waxes. Compound lipids are esters of fatty acids containing groups in addition to an alcohol and a fatty acid. They include phospholipids, cerebroside and other compound lipids. Derived lipids are substances derived from the above mentioned groups by hydrolysis. They include fatty acids (saturated and unsaturated), glycerols and steroids.

CHOLESTEROL

Cholesterol is a complex monohydric secondary alcohol, stable white crystalline substance, insoluble in water but readily soluble in non polar solvents. Chemically the basic structure consists of 27 carbon

atom rings referred to as cyclopentanoperhydrophenanthrene ring. It is widely distributed in all cells of the body. The total cholesterol content of the body is about 200 mg/kg of body weight (Bell et al, 1972). Two thirds of cholesterol in blood is found in esterified form, while one third is present as free cholesterol (Fredrickson, et al, 1967). Cholesterol is only present in foods of animal origin. Greater part of it is produced in the body by synthesis which takes place in liver, adrenal cortex, skin, intestines and testes.

CHOLESTEROL METABOLISM

Most of tissue primarily of skin and intestine have the capacity to synthesize cholesterol(Srere et al, 1950). The rate of cholesterol synthesis is high in liver, intestine and skin and accounts for as much as 90% of total body cholesterol production, while it is low in other body tissues.

Dietary cholesterol is absorbed in intestine in company with other lipids and is incorporated in chylomicrons and very low density lipoproteins (VLDL). 80-90% of cholesterol absorbed undergoes esterification, which may take place in intestinal mucosa. Chylomicrons deliver their cholesterol to liver from where it is transported to plasma in the form of VLDL which ultimately changes to low density lipoproteins(LDL)(Conner, 1979).

Most of the plasma cholesterol is esterified and is present in LDL (75-80%) which is converted to VLDL by progressive reduction of lipoproteins and change in apoproteins.

PLASMA TRIGLYCERIDE AND CHOLESTEROL TRANSPORT

Ultimately LDL is taken up and broken down by extrahepatic parenchymal tissues and cholesterol is delivered to cells.

Sequence of events proposed are that cells possess surface receptors which especially bind LDL. This interaction between the LDL and its specific receptor results in delivery of lipoprotein to cell interior where both proteins and cholesterol ester are hydrolysed by acid hydrolysis(Brown and Goldstein, 1976). Cellular cholesterol synthesis is influenced by the lipid composition of the medium. The main enzyme in this process is "3 hydroxy 3 methyl glutaryl coenzyme - A reductase" which can be induced by cholesterol deprivation.

EXCRETION

It occurs mainly by 2 pathways one being conversion to bile acid and its excretion while the other being excretion of neutral sterol in faeces. Liver is the main organ for cholesterol disposal. Before elimination, cholesterol must enter liver and be excreted in bile

either as cholesterol or cholic acid. Loss through urine is negligible..

TRIGLYCERIDES

Triglycerides (Triacylglycerols) are esters of fatty acids and glycerol. Human adipose tissue consists chiefly of triacylglycerols regardless of anatomical location. Triglycerides form the largest portion of dietary fat.

METABOLISM

Triglyceride metabolism is said to have two pathways namely exogenous and endogenous pathway.

Exogenous Pathway of Triglyceride Metabolism (Fredrickson et al, 1967).

In adults 1-2 gm/kg of triglycerides are ingested daily (Henry, 1977). From the intestinal mucosa they are largely absorbed as chylomicrons and reach the blood through thoracic duct. These chylomicrons are taken to adipose tissue and skeletal muscles, and then triglycerides are hydrolysed there to release free fatty acids and monoglycerides. Free fatty acids are either re-esterified and stored in the adipose tissue and skeletal muscles or oxidized (Havel, 1961).

Endogenous Pathway of Triglyceride Metabolism

Endogenous hyperlipidemia perhaps first became a distinctly recognizable phenomenon, when Watkin et al

(1950) noted that fat free high carbohydrate diet increased the triglyceride concentration.

Liver is considered as the major site for endogenous triglyceride metabolism (Havel, 1962 and Goldstein, 1961). An important source of fatty acids needed for triglyceride synthesis is the plasma free fatty acid. The flux of the free fatty acids into the liver, heart, and skeletal muscles, is governed by their rate of release from adipose tissue. Any factor that increases lipolysis or decreases glycerol esterification in the adipose tissue causes outpouring of free fatty acid (Steinberg and Vaughan, 1965). Much of this free fatty acids is removed by liver and excess beyond what it can use or store is resynthesized into glycerides and resecreted as VLDL (Havel, 1957). VLDL is the chief triglyceride binding lipoprotein present in the plasma. VLDL particles carry 5-10 times triglycerides than cholesterol esters.

LIPOPROTEINS

These are globulin particles of high molecular weight and transport non polar lipids (Cholesterol and triglycerides) through plasma. Each lipoprotein particles contains a non polar core, comprising of triglycerides and cholesterol in varying proportions. Surrounding the core is a polar surface coat of phospholipids that stabilize the lipoprotein particles, so that is can remain

insoluble in the plasma. Surface coat consists of phospholipids, esterified cholesterol and apoproteins. The proportions of these lipids and proteins however, differ greatly resulting in differences in physiochemical properties which permit their separation (Chait A, 1978).

Boydd,Nobbe and Schettler (1967) have classified lipoproteins into 4 major classes that are normally present in the plasma.

CLASSES OF LIPOPROTEINS

	Chylomicron	VLDL	LDL	HDL
Specific Gravity	0.94-0.98	0.98 - 1.006	1.006- 1.063	1.063- 1.21
Approx. diameter(A^{o})	800-5000	300-800	180- 28	50-120
Molecular weight	4×10^{10}	6×10^{6}	2×10^{6}	$2x10^{5}$
Chemical composition(%)				
Triglyceride	85	50	11	8
Cholesterol	2	7	8	3
Esterified cholesterol	5	12	37	14
NEFA	-	2	1	3
Phospholipids	6	18	22	22
Proteins	2	10	21	50
Apoproteins	A_1, A_2, C_1, C_2, C_3	B, C_1, C_2 C_3, E	B	A_1, A_2

(From Boyd, Nobbe and Scheitler , 1967).

VERY LOW DENSITY LIPOPROTEINS (VLDL)

It is heterogenous group of large molecules rich in triglycerides. It also contains free cholesterol cholesterol ester, and phospholipids. The main source of VLDL is liver, however, it is also produced in the gut in nascent form. It is ia vehicle for transport of triglycerides from liver to extrahepatic tissues.

Intestinal VLDL differs from hepatic VLDL by apoprotein C, which is not synthesized in the intestine cells. Nascent VLDL is rapidly converted to mature form by acquiring apoprotein C from HDL, VLDL of hepatic origin contains triglyceride of endogenous origin. Normal life span of VLDL in plasma is about 6-12 hours. Conversion of VLDL to LDL involves a very substantial remodelling of the large particles in which all components other than the beta apoprotein are lost to a lesser or greater extent. VLDL transformation appears to be attributable mainly to lipoprotein lipase rather hepatic lipase.

LOW DENSITY LIPOPROTEINS (LDL)

Most of plasma LDL is derived from catabolism of VLDL, much of which occurs within the intravascular compartment. Particles remaining after the intravascular catabolism of VLDL and chylomicrons are enriched in aproprotein beta and cholesterol ester. The direct contribution of chylomicrons to circulating LDL is uncertain. LDL is also formed as a result of reaction between liver and chylomicron remnants.

Core of LDL is almost entirely of cholesterol ester and surface coat contains only apoprotein beta. About 3/4 of total serum cholesterol is within LDL particles.

Liver has long been assumed to be involved in LDL uptake and metabolism. Much of LDL uptake by liver is of extrahepatic origin. The liver may possess high affinity receptor for LDL, though this tissue does not take up the cholesterol of LDL nearly as effectively as it does the cholesterol from the chylomicron remnants.

The main function of LDL is to supply cholesterol to a variety of extrahepatic parenchymatous cells such as adrenal cortical cells, muscle cells, lymphocytes and renal cells. These cells have LDL receptors.

LDL receptors regulate the LDL catabolism and cholesterol synthesis. Number of surface receptors for LDL depends upon its cholesterol requirements.

HIGH DENSITY LIPOPROTEIN (HDL)

They are lipid fraction complexes defined by floatation in ultracentrifuge between density 1.063 and 1.21 gm/ml. due to presence of major protein constituents (Apo-protein A_1 & A_2) and by alphamigration on electrophoresis.

The bulk of HDL masses appear to arise from interaction of precursor particles (nascent HDL) secreted by the liver and intestines with lipids and proteins released during the catabolism of triglyceride rich lipoproteins. A portion of HDL also arises from transfer

and uptake of lipid particularly free cholesterol from cell membranes.

The major function of HDL appears to be a direct role in the centripetal transport of cholesterol from peripheral tissues to liver (Glomset, 1968) and in direct role in facilitating triglyceride transport by transferring aporpotein C to VLDL and chylomicron.

METABOLISM IN FETUS

Before birth the fetus utilizes carbohydrates as the major fuel for energy production. This correlates well with the observation that the respiratory quotient (R.Q.) in fetus and at birth is one (James, 1972). In utero transfer of glucose occurs through placenta. The fetal glucose concentration followes the maternal closely, although it is generally somewhat lower. The net transfer is towards fetal side. Free fatty acids have been shown to cross placenta but there is little or no transfer from mother to fetus of cholesterol, triglycerides or phospholipids (Forfar and Arneil, 1984). The synthesis of lipids in fetus proceed from glucose and fatty acid precursors in early stages of gestation, and lipid content in fetus increase to 300 fold from first month to ninth month of gestation (Roux et al, 1974). After birth with cutting off of the nutrients from the maternal circulation, and before milk feeding is started the newborn has to depend on its own endogenous sources of nutrients for survival.

As the carbohydrate (glycogen) stores of body are meagre and protein metabolism can account for only a fraction of the total energy requirements, body lipids become a major source of energy for the newly born infant. Increased mobilisation of lipids from the stores and increased lipolysis in the immediate post natal period leads to a rise in the levels of total lipids, cholesterol, phospholipids and free fatty acids (Brown et al, 1939; Van Duyne, 1959; Persson, 1956 and Christensen, 1974). The mechanism for the oxidation of fatty acids rapidly increases in activity after birth (Forfar, Arneil, 1984).

However, information on this aspect of lipid metabolism is meagre in the case of prematures and newborns with intrauterine growth retardation, as is evident from a brief review of the proceedings so far on this aspect.

It was some 78 years ago when cord blood lipids were measured for the first time by Hermann and Newmann (1912) when they performed a study on 30 normal deliveries and recorded the maternal and cord blood cholesterol levels with the aim to find out the normal values and any relationship between the two values. They observed that the cord blood cholesterol (62 mg/dl) values were considerably lower in comparison to their maternal counterpart (mean value 264 mg/dl).

Gyorgy (1924) observed 6 cases and recorded the cord blood cholesterol and lipid phosphorous values and

compared them with the maternal serum counterparts. He chose 6 normal full term healthy deliveries which formed his study group. He observed that the mean values of serum cholesterol in cord and maternal blood were 69 mg/dl and 255 mg/dl, while those of lipid phosphorous were 3.8 mg/dl and 9.3 mg/dl respectively. These results showed that the observed values of cord blood cholesterol were comparable with those observed earlier and at the same time were less in comparison to maternal serum values this finding also supported those by earlier workers. The serum lipid phosphorous values in cord blood were less in comparison to maternal serum lipid phosphorous values.

Gordon and Cohn (1928) conducted a study on 16 full term normally delivered cases to record their cord blood cholesterol levels and lipid phosphorous levels. They observed that the mean cord cholesterol values were 89 mg/dl which were slightly higher than those recorded by earlier workers, while the mean cord lipid phosphorous values were 4.1 mg/dl which were comparable to those observed by other workers. Here the aim was to establish the normal value and no correlation with maternal values was considered.

Sperry (1936) observed cholesterol values in cord blood of 7 neonates and found that the mean values were 61 mg/dl which were in close approximation to those obtained earlier.

Sadowsky et al (1947) performed a study on 14 Israeli neonates who were delivered normally and observed the cord and maternal cholesterol values. In their study, they found the mean cord blood cholesterol value to be 107 mg/dl which was higher than those observed by earlier workers, while the maternal mean blood cholesterol values were 262 mg/dl which were comparable to earlier obtained values.

Rafstedt et al (1954) studied 32 neonates to observe the cord blood cholesterol and lipid phosphorous values. The mean cord blood cholesterol value was observed to be 67 mg/dl which was consistent with other observations, while the mean lipid phosphorous values were 4.8 mg/dl which also was in close proximity to those obtained by other workers. In their study Rafstedt et al reported that cord plasma lipid concentrations were the same in low birth weight babies as compared to those of normal birth weight but this data was limited to cholesterol and total lipid concentrations, and the low birth weight babies were not subdivided into preterm and small for date groups.

Russ et al (1954) have determined lipoprotein, cholesterol and phospholipid content in mothers and their newborn infants. They recorded mean cord blood cholesterol values as 68 mg/dl while the mean maternal values were 282 mg/dl. These values correlated well with those observed in the past.

Brown et al (1959) performed a study on 50 neonates and their mothers to determine normal values of various serum components including proteins, lipoproteins and lipids at birth and their relation with corresponding maternal values. The maternal venous blood samples were collected during I stage of labour and cord blood samples were collected just after birth. The mothers chosen underwent normal full term labour. The protein levels in maternal blood were 6.32±0.52gm%, 2.27±0.33 gm% for total and albumin respectively and were 6.13±0.64 gm% and 3.05±0.45 gm% respectively in the cord blood samples for the same. The maternal values were 1104±172 mg%, 257±71 mg%, 847±176 mg% and 273±52 mg% for total lipids, lipoprotein lipids, betalipoprotein lipids, and cholesterol respectively, while they were 371±75 mg%, 147±40 mg%, 224±41 mg% and 82±17 mg% respectively for the same in cord blood samples. These findings agreed closely with those of Rafstedt et al (1954) and Russ et al (1954).

Brody and Carlson (1962) included 52 Swedish cases in their study and observed the cord blood cholesterol, triglyceride and lipid phosphorous values. They included full term deliveries in their study. Their aims were to observe the normal cord blood cholesterol value and its relation with those observed in past as well as to observed the normal value of cord blood triglyceride levels. The mean cord blood cholesterol level observed by these authors was 66 mg/dl, cord triglyceride level

was 34 mg/dl (0.38 m mol/l) and lipid phosphorous level was 4.2 mg/dl. The levels of cholesterol and lipid phosphorous were in proportion to previous recordings.

The observations of Kleeberg and Polishuk (1963) of 129 cases have revealed mean cord and maternal blood cholesterol value of 66 mg/dl and 261 mg/dl respectively. Both these observations have been a confirmation of the past recordings. In fact it was the largest study till date, but it did not consider gestational age or birth weight as the further differentiating criteria.

Kaplan and Lee (1965) undertook a study on 56 cases and collected maternal and cord blood samples from 56 unselected pregnancy cases, at the time of delivery. Later infants blood sample was also collected and cholesterol, triglycerides, and lipid phosphorous were estimated.

The mean cholesterol concentration was 95 ± 18 mg/dl in cord blood and 264 ± 56 mg/dl in maternal blood, mean triglyceride concentration was 34 ± 14 mg/dl in cord blood and 159 ± 54 in maternal blood, while mean lipid phosphorous value was 5.3 ± 1.0 mg/dl in cord blood and 12.9 ± 2.4 mg/dl in maternal blood.

This study confirmed the finding of Brody and Carlson (1962) that the concentration of serum triglycerides is quite low in the cord blood and newborn. Employing a method similar to theirs for the estimation

of this constituent, it was found that the level of triglyceride was same in both studies while there was a significant difference in the cholesterol levels. The mean cholesterol level in cord blood was 95 mg% in contrast to 67 mg/dl and 66 mg/dl as observed in studies by Rafstedt et al (1954) and Brody and Carlson (1962).

This study also demonstrated that cord blood triglycerides were lower in cord blood in comparison to maternal values. The authors had ascribed the differences in the maternal and cord blood lipid values to failure of lipids to pass through the placental barrier. They also considered differences in fetal and adult metabolism to be responsible for the same, or perhaps the quiescent state of fat utilization in the fetus and the absence of a need for fat mobilization may also be responsible for low serum triglyceride concentration in the cord blood samples.

Darmandy et al (1972) worked on 302 cases and conducted a prospective study of serum lipids in infants throughout first year of life. The purpose of the study was to establish the relationship between cord serum cholesterol levels and the values subsequently achieved in individual babies, to determine whether a diagnosis of hypercholesterolemia could be made with certainty during the first year of life. In addition normal values for this period were also obtained. Cord blood was obtained from 302 full term babies, just after birth.

Later blood samples were collected by capillary puncture from heel or finger at 1 week, 6 weeks, 4 months, 8 months and 1 year of age and cholesterol values were estimated.

The mean cholesterol values observed by these workers were 78±23 mg/dl, 155±31 mg/dl, 155±31 mg/dl, 184±36 mg/dl, 195±37 mg/dl, 191±36 mg/dl in cord blood, at 1 week, 6 weeks, 4 months, 8 months and 1 year of age respectively. Only one case had cord cholesterol value of more than 100 mg/dl. The values for females were on a higher side than males. Out of 274 cases studied at 1 yesr age, 24 had cholesterol values of more than 240 mg/dl, out of which 23 cases had positive family history of hypercholesterolemia. The workers suggested that cholesterol values at 1 year age were a more reliable indicator of familial hyperlipidemia in comparison to cord cholesterol values.

Fosbrooke et al (1973) conducted a study to elucidate the effects of gestational age and nutritional status on the concentration and composition of cord blood lipids, so as to obtain the evidence concerning intrauterine fat metabolism. Plasma lipid concentration and fatty acid composition were determined in the cord blood of three groups of babies. The first group was of low birth weight preterm babies, the second group included full term low birth weight babies and the third group (reference group) included full term normal weight babies.

The values of cord blood cholesterol derived at this study were 96.3±18.7 mg/dl for preterm(less than 37 weeks), 97.2±31.5 mg/dl for term babies (37.41 weeks) and 82.4±22.9 mg/dl for light for date babies(37-41 weeks) while at the same time the cord blood triglyceride levels were 19.8±7.8 mg/dl, 29.6±12.0 mg/dl and 45.4±28.2 mg/dl respectively in the above mentioned groups. The total concentration of cholesterol did not vary much between these groups. Triglyceride levels were higher in the term and was highest in the light for date babies. There was a significant correlation between the triglyceride concentration of the appropriate weight babies and gestational age, but inspection of individual values showed that the concentration varied little before 37 weeks and then increased substantially in babies born after 37 weeks of gestation. Triglyceride concentration in the 'light for date' group were higher than in the appropriate weight babies. It was also observed that in babies delivered beyond 28 weeks of gestational age, the cholesterol concentration were not related to gestational age or nutritional status.

The lower triglyceride concentration in the preterm infant reflected lesser importance of fat metabolism earlier in pregnancy and as deposition of fat in adipose tissue took place mainly in the last month of pregnancy. The higher triglyceride concentration in the light for date infants were compared by authors to those

found in marasmic children due to malnutrition developing post natally. The authors related them with mobilisation of intrauterine fetal adipose stores in response to intrauterine malnutrition.

Tsang et al (1974) focussed their attention on neonates found to have elevated cord triglyceride levels during a survey of 2000 consecutive unselected live births.

They selected 60 infsnts by choleseterol screening programme using parental cholesterol triglyceride values and judging them against the normal ones as suggested by Fredrickson and Levy (1972). Factors such as maternal hypertension, diabetes, prolonged labour, prolonged rupture of membranes, malpresentation, gestational age, post term delivery, low Apgar score were not considered while selecting the cases.

57 infants were selected in another group in which one of the parents had hypertriglyceridemia with normal to slightly elevated cholesterol levels. The above mentioned maternofetal factors were not considered while selecting the cases. This group was used as a second control in addition to former group of infants, as it represented a group with some genetic potential for eventual development of hypertriglyceridemia.

In the distribution curve the 95th percentile value for cord triglyceride was determined as 70 mg/dl and this was taken as a cut off between normal and elevated levels.

In 60 neonates born to parents with normal cholesterol and triglyceride values, mean cord blood triglyceride levels were 36±18 mg/dl with a range from 8-95 mg/dl. In 57 neonates born to parents where one of them had hypertriglyceridemia, the mean cord triglyceride levels were 37±19 mg/dl. Both the above mentioned control groups had values comparable with each other.

In 2000 consecutive live births, 56 neonates had cord triglyceride levels more than 70 mg/dl(cut off point) mean value being 111±33 mg/dl, and the range being 71 to 218 mg/dl; 46 normal neonates with cord triglyceride levels less than 70 mg/dl, mean values 30±16 mg/dl and range 1-70 mg/dl, were selected as controls. These two groups were estimated against maternofetal factors, and cord triglyceride levels were observed to be related with perinatal stress factors.

In the group of hypertriglyceridemic neonates (56 cases) 36% (20/56) cases had maternal hypertension, 21% (12/56) cases had prolonged labour, 21% (12/56) had cord around neck, 43% (25/56) had meconium stained amniotic fluid, 54%(30/56) were post term deliveries and 36%(20/56) had decreased 1 minute Apgar score while in the control group (46 cases) the incidence of the above mentioned perinatal stress factors was 4.3%(2/46), 0% 0(0/46), 2.2%(1/46), 11%(5/46), 6.5%(3/46), and 2.2% (1/46) respectively. There was no association between triglyceride levels and maternal diabetes, prolonged

rupture of membranes, caessarean section, abnormal presentations, sex of child, low birth weight for gestation (small for date). There was a linear relationship between total number of significant perinatal factors and cord triglyceride levels. There was also an inverse relationship between cord blood triglyceride levels in 102 neonates (56 cases and 46 controls) and one minute Apgar score.

The authors suggested that stress in utero or birth canal, or anoxia may lead to early mobilization and depletion of glycogen stores and an early conversion to oxidation of fats, there by causing hypertriglyceridemia in neonates affected with perinatal factors leading to stress. The authors suggested cord blood hypertriglyceridemia as a useful indicator of antepartum or intrapartum fetal stress or compromise.

Desai et al (1977) studied and analysed 113 full term newborns, delivered by normal labour following uncomplicated pregnancy. The mean birth weight was 2800 gm with a range of 2500 to 3600 gm. The cord cholesterol values ranged between 35-128 mg/dl and phospholipids between 58-160 mg/dl, while the mean values were 79 ± 17 mg/dl, 62 ± 21 mg/dl and 95 ± 17 mg/dl respectively.

The levels of cholesterol in this study were in agreement with previous recordings while the level of triglycerides observed in this study was higher than those obtained earlier by Western authors.

Cress et al (1977) observed 275 neonates for cord blood cholesterol and triglyceride levels and noted cord blood cholesterol level as 70±17 mg/dl with a range of 30-153 mg/dl. The 95th percentile value was 105mg/dl. Mean cord blood triglyceride levels were 33±26 mg/dl.with range of 5-192 mg/dl. The 95th percentile value for cord blood triglyceride was 77 mg/dl. They observed that out of 22 neonates whose cord blood lipid values exceeded the 95th percentile values, 9 had hypercholesterolemia and 13 had hypertriglyceridemia. Four neonates had elevated values both cholesterol and triglyceride range 103-120 mg/dl and 68-137 mg/dl respectively.

None of the 275 neonatal sera had demonstrable amounts of IgA antibody, indicating that there was no maternal contribution to the c ord blood samples.

Post term delivery (741 weeks) was seen in 20%(3/15) of newborn with increased cholesterol levels 16%(3/19) of neonates with increased triglyceride level and 8%(5/65) of neonates with normal values.

One minute Apgar score of 6 or less was seen in 7%(1/15) of cases with hypercholesterolemia and 37%(7/19) of neonates with hypertriglyceridemia.

Resuscitation was required in 20%(3/15) of hypercholesterolemic neonates, and in 21%(4/19) of hypertriglyceridemic neonates, as compared with 12% (8/65) of normal controls.

Prolonged labour (715 hours) was observed in 13%(2/15% of infants with elevated choleseterol levels

and in 11%(2/19) of infants with high triglyceride levels as compared with 9% (6/65%) of normal infants.

Maternal hypertension was present in mothers of 7%(1/15) of hypercholesterolemic infants, and 21% (4/19) of hypertriglyceridemic neonates as compared to 1.5% (1/65) of mothers of normalipidemic infants.

In this study it was seen that high cord blood cholesterol or triglyceride values were associated with maternal fetal problems related with unfavourable intra-uterine environment, fetal distress, fetal anoxia. There was a significant correlation between post term delivery and hypercholesterolemic neonates and low Apgar scores along with maternal hypertension were more associated with hypertriglyceridemia. Low cord blood lipid levels were seen after an uneventful pregnancy, with the Apgar scores greater than 8.

The authors stated that during birth newborn entered from a warm intrauterine environemtn to unpleasant cool atmosphere and during this period of adjustment the energy requirements were provided by utilisation of carbohydrate and fat stores. The pituitary adrenocortical axis was supposed to be capable of stimulating fetal lipogenesis at term, and during stress of delivery catecholamines elicited an immesiate response on adipose tissue. Neonatal stress associated with maternofetal perinatal problems especially maternal hypertension post term delivery. Low Apgar score were related to elevated cord blood cholesterol and triglyceride levels.

Prakash et al (1980) studied 50 newborns for serial estimation of free fatty acids. They included cases where the delivery was normal vaginal and mothers were sure of their first day of last menstrual period (LMP). Later on, the gestational age was also assessed postnatally by Dubowitz's method. They classified 50 neonates into 3 groups on the basis of gestational age and birth weight, as follows :- control groups of full term appropriate for date babies; small for gestational age group who were full term and weighed less than 2000 gms and pre term group who were born before 37 weeks of gestational age.

They observed the maternal values to be higher in all of above mentioned 3 groups than cord blood values. The cord blood levels of free fatty acids were higher (mean value 8.40 ± 0.14 m eq/l) in small for gestational age group, than control (mean value 0.38 ± 0.12 m Eq/l) and pre term group (mean value 0.35 ± 0.12 m Eq/l). This difference was not significant ($p > 0.05$). However, significantly low values were observed in pre term babies in comparison to control group ($p < 0.01$) at 3 hours of age.

The free fatty acid levels at various gestational ages were as follows; upto 32 weeks, the cord blood levels were significantly lower (0.26 ± 0.06 m Eq/l) in comparison to 33-36 weeks (0.35 ± 0.07 m Eq/l) ($p < 0.05$). After 36 weeks levels did not differe significantly.

The authors expressed that the early rise in free fatty acid levels after birth was due to lipolysis and release of free fatty acid from adipose tissue during course of utilisation for energy purposes.

A study was carried out on 57 neonates for cord blood lipid profile by Sharma et al (1983). They divided the 57 cases into 3 groups on the basis of gestational age and birth weight. The first group included normal term appropriate for gestational age newborns(31), the second group included full term small for gestational age infants (12) and the third group included pre term appropriate for gestational age infants(14). The classification was done according to Lubschenco et al (1963). The total lipids, cholesterol and free fatty acids in cord blood were estimated.

The cord cholesterol levels in normal full term small for date and pre term were 74±17 mg/dl, 64.8±12.3 mg/dl and 64±13 mg/dl respectively while the cord blood phospholipid values were 112±36.3 mg/dl, 101.6±30 mg/dl, and 130±11 mg/dl respectively in above mentioned groups. The free fatty acid levels in cord blood were 0.38±0.03 m mol/l, 0.29±0.06 m mol/l and 0.26±0.06 m mol/l in normal full term, small for date and pre term groups respectively.

The levels of various lipid fractions in cord blood were seen to be lower in small for gestational age group (second group) and pre term group (third group) as

compared to healthy full term neonates (first group) and the difference was statistically significant only with free fatty acid levels ($p < 0.001$), and attributed this to lower fat stores in small for gestational age infants in comparison to full term healthy neonates. The lower values in pre term infants were attributed probably to a possible quantitative or qualitative deficiency of the enzyme lipoprotein lipase, which was responsible for release of free fatty acid from neutral fats (triglycerides). The lower levels of enzyme have also been reported by Sigmura et al (1974) and Prakash et al (1980).

Haridas and Acharya (1984) conducted a study on 180 newborns and their mothers, who belonged to low socioeconomic strata. This study consisted of determination of cholesterol and triglyceride values in cord blood and serum on 2nd, 3rd, 4th and 5th day in normal full term, preterm, and low birth weight babies along with maternal blood values.

The mean cord blood triglyceride values were 45 ± 13.8 mg/dl (range 20-89 mg/dl), 59 ± 22.3 mg/dl(range 24-119 mg/dl), and 56 ± 16.1 mg/dl (range 22-95 mg/dl) in normal full term, preterm infants and small for date babies respectively. The triglyceride values were 157 ± 48.9 mg/dl (range 65-317 mg/dl), 135 ± 26.2 mg/dl (range 87-190 mg/dl) and 114 ± 41.2 mg/dl (range 71-343 mg/dl) respectively in mothers of above mentioned group infants.

The mean cholesterol values were 90 ± 17.7 mg/dl, (range 55-125 mg/dl), 95 ± 20.2 mg/dl (range 42-130 mg/dl)

and 91±20.2 mg/dl (range 42-122 mg/dl) in cord blood of normal full term, preterm and small for date infants, while they were 230±34.9 mg/dl(range 120-330 mg/dl), 228±37.6 mg/dl (range 143-292 mg/dl) and 233±32.8 mg/dl (range 167-320 mg/dl) respectively in the mothers of the above mentioned infant groups.

The mean triglyceride values were 65±13.8 mg/dl and 89±17.1 mg/dl in normal full term infants on 2nd/3rd and 4th/5th day respectively. These values were 72±16.3 mg/dl and 95±17.1 mg/dl in preterm infants on above mentioned days respectively. The levels were 74±15.3 mg/dl and 93±16.4 mg/dl in small for date babies on the above mentioned days respectively. The cholesterol levels were 124±9.6 mg/dl, 156±9.6 mg/dl in normal full term, 119±11.6 mg/dl and 143±8.7 mg/dl in pre term, and 122±7.2 mg/dl and 143±7.6 mg/dl in small for date babies on 2nd/3rd and 4th/5th day respectively.

The neonatal cholesterol and triglyceride values were lower than maternal counterparts. The cord blood cholesterol levels were not significantly different in the three groups but by the 4th/5th day normal full term babies exhibited higher values. The low birth weight and pre term infants had higher triglyceride values in cord blood than normal full term. The levels continued to be higher in low birth weight babies and pre term infants but the differences were statistically insignificant.

The difference in cord and maternal lipid values revealed lack of maternal contribution to fetal lipids. The authors stated that low birth weight infants were born with intrauterine malnutrition which favoured adipose tissue breakdown and liberation of free fatty acids. The portion of free fatty acid escaping oxidation for energy production were synthesized into triglycerides in the l liver which lead to increased values of triglyceride in cord blood of low birth weight babies. The raised triglyceride levels in post natal period was due to utilisation of adipose tissue for energy requirements as glucose was conserved for energy requirements of brain and erythrocytes. This liberated free fatty acids which lead to triglyceride synthesis and raised triglyceride levels. The post natal elevation in cholesterol levels was due to its enhanced synthesis as a result of increased enzyme and substrates required for cholesterol biosynthesis.

Mathur et al (1986) have done a study on 56 newborns for cord blood cholesterol values. These neonates were delivered to healthy mothers. Their gestational age was determined by Dubowitz's criteria. In this study out of 56 neonates, 14 were pre term and 42 were term babies. The cord blood was collected from the placental end just after delivery.

They observed mean cord blood cholesterol values to be as 105.27$\pm$17.14 mg/dl with a range of 70-135 mg/dl

The mean cord blood values in pre term babies were 92.57± 14.94 mg/dl as compared to 112.2±14.58 mg/dl in term babies. In 18 babies weighing less than 2.5 kg the mean values were 93.67±14.64 mg/dl. While in 38 neonates weighing 2.5 kg or more the mean values were 110.76±15.46 mg/dl. A positive correlation was found between birth weight and cord blood cholesterol levels in this study.

Kumar et al (1989) undertook a study to find out the influence of prematurity and/or growth retardation on the cord lipid levels. They included seventy three (73) newborns, delivered to healthy mothers with uncomplicated pregnancy and labour. The cord was cut and clamped within 3 minutes of delivery but prior to delivery of placenta. Mixed arterial and venous blood wasx allowed to flow freely and contamination with maternal blood was avoided. The gestational age was assessed by first day of last menstrual period, supplemented with clinical evaluation by Ballard Seore (1977). The newborns were divided into 4 groups on the basis of gestational age and birth weight. First group included full term appropriate for gestational age babies; the second group included full term small for gestational age babies, the third group included pre term appropriate for gestational age babies and the fourth group included preterm small for gestational age babies. The cord blood cholesterol triglyceride and free fatty acid values were determined.

Out of 73 neonates, 29 belonged to first group, 17 belonged to second group, 22 belonged to thimd group and 5 were of fourth group. The mean cord blood cholesterol values were 85.83±22.85 mg/dl for first group, 84.35±17.15 mg/dl for second group, 100.09±32.19 mg/dl for third group and 92.20±8.32 mg/dl for fourth group. These values showed no significant difference.

The mean cord blood triglyceride values were 35.27±17.49 mg/dl, 55.34±23.95 mg/dl, 70.67±32.68 mg/dl and 104.50±20.80 mg/dl for above mentioned groups respectively. These values were significantly higher in the full term small for gestational age and pre term small for gestational age groups, in comparison to their appropriate for gestational age counterparts.

The free fatty acid levels in various groups of neonates were 0.27±0.14 m mol/l, 0.25±0.10 m mol/l, 0.31 ±0.11 m mol/l, and 0.41±0.07 m mol/l in full term appropriate for gestational age, full term small for gestational age, pre term appropriate for gestational age and pre term small for gestational age group respectively and showed that free fatty acid levels were significantly higher in pre term small for gestational age group infants.

The workers concluded that cord blood cholesterol levels were not influenced by birth weight gestational age, and elevated cholesterol levels may indicate hypercholesterolemia. On the other hand the levels of triglycerides and free fatty acids were affected by birth weight and

gestation, and an infant should not be labelled as hyperlipidemic unless these factors were considered.

The authors stated that stress in any form has been shown to raise serum triglyceride levels. As per term delivery was not a normal phenomenon and it involved some amount of stress to fetus which could or could not manifest clinically. Small for gestational age babies were born with intrauterine malnutrition which favoured adipose tissue breakdown, liberating free fatty acids. The portion of free fatty acids which escaped oxidation for energy, was converted to triglyceride in the liver, thereby resulting in increased triglyceride levels.

Lakhtakia et al (1990) performed a study on 100 neonates to detect the effect of familial hypertension on the cord blood cholesterol and triglyceride levels. They selected 50 cases where there was family history of essential hypertension (in mother, grand parents or in other siblings) and 50 control subjects who were full term delivered neonates, after normal labours without any adverse fetomaternal factors, born in a family with no history of ischaemic heart disease, hypertension or diabetes mellitus.

The mean$\pm$S.D. cholesterol values in cord blood of neonates with family history of hypertension with the involvement of parents, grand parents, and siblings of parents were 123.24$\pm$22.32 mg/dl, 93.7$\pm$16.96 mg/dl and 88.0$\pm$11.95 mg/dl respectively. The cord triglyceride

levels in the above mentioned groups were 58.16±17.52 mg/dl 33.94±16.7 mg/dl and 30.0±13.16 mg/dl respectively.

The mean serum cholesterol levels in the study and control group were 108.92±26.25 mg/dl and 86.84±26.62 mg/dl respectively. The mean triglyceride levels in the above said groups were 45.52±20.89 mg/dl and 28.72±17.81 mg/dl respectively. The differences in the study and control groups regarding the cholesterol and triglyceride levels were highly significant ($p < 0.001$).

On observing elevated cholesterol and triglyceride levels in neonates born with family history of hypertension, they concluded that genetic factors may play a significant role in transmission of hyperlipidemia as seen in neonates of hypertensive families. They labelled these cases as high risk cases and suggested periodic surveillance for atherosclerotic changes.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried out in the Department of Paediatrics, in active collaboration with the department of Biochemistry and department of Obstetrics and Gynaecology, Maharani Laxmi Bai Medical College, Jhansi. Babies delivered by normal vaginal delivery in the labour room of Obstetrics and Gynaecology department, between March, 1990 and November, 1990 were included in the study. Babies born by Caessarean section were not included in the present study.

STUDY GROUP

The study group consisted of 51 new born babies delivered by normal vaginal delivery. Infants with still birth were excluded from the present study.

New born babies were classified according to their gestational age and birth weight by the criteria laid down by Singh et al (1974). On the basis of gestational age, babies were divided into 3 subgroups, preterm babies($\angle$37 weeks), term babies (37-41 weeks) and post term babies ($\nearrow$41 weeks).

According to birth weight babies were further classified as follows by ascertaining their position on intrauterine growth curve (Singh et al, 1974).

1. Pre term (gestational age less than 37 weeks).
   a. Small for gestational age (SGA)(birth weight below 2 S.D.).

b. Appropriate for gestational age (AGA) (birth weight between +1 S.D. & - S.D.).

2. Term babies (gestational age 37-41 weeks).
   a. Small for gestational age (birth weight less than 2 S.D.).
   b. Appropriate for gestational age (AGA) (birth weight between + 1 S.D. and - 1 S.D.).
3. Post term babies (gestational age 42 weeks or more).
   a. Small for gestational age (birth weight below 2 S.D.).
   b. Appropriate for gestational age (birth weight between + 1 S.D. and -1 S.D.).

The definition for the low birth weight babies as laid down by World Health Organisation (WHO, 1961) was adopted in the present study to classify the babies with birth weight less than 2,500 gms in low birth weight (LBW) group.

OBSTETRICAL AND PAST HISTORY

Apart from taking the history of socio-economic status, detailed obstetrical history was also taken into account. History regarding the parity, abortions, previous premature births, still birth, neonatal death were recorded in each case. Application of forceps at the time of previous deliveries and deliveries by LSCS were also recorded.

Emphasis was given in each case to record the history of last menstrual period and was recorded when the mother was sure of it. Gestational age was calculated in complete weeks from first day of last menstrual period and by the physical and neurological criteria laid down by Dubowitz et al (1970).

ANTENATAL, NATAL AND POSTNATAL HISTORY

A detailed history of any medical or surgical disorder viz. anemia, convulsions, oedema, hypertension, cardiac disorder, diabetes, antepartum haemorrhage, exanthematous fever, syphillis, gonorrhoea was recorded. History of drug intake and addiction to narcotics, smoking etc. were also taken in each case. Multiple pregnancies were also considered in the history.

History was taken regarding the mode of delivery duration of labour, leaking P/V, meconium staining of liqour, cry and activity of child after birth and cyanosis after birth, to rule out any evidence of perinatal stress.

EXAMINATION OF NEW BORN

Apgar scoring of child was done at 1 minute and 5 minutes to detect any evidence of birth anoxia. After the birth of child, color, heart rate, respiration, response to nasal catheterisation, cry, activity and tone were recorded on a predesigned proforma to assess the Apgar score.

Thorough clinical examination was done in each case. Head of the newborn baby was examined in detail for the size of fontanelle, over riding of skullbones, moulding, presence of caput succedaeneum, cephalhaematoma shape of head and any mark of injury over head. Eyes were examined for any evidence of conjunctivitis or cataract. Detailed examination was done to find out any congenital abnormality. A thorough systemic examination of cardiovascular system, respiratory system, nervous system and abdomen was also done in each case.

Anthropometric measurements viz. head circumference, chest circumference, length were recorded in the proforma. Birth weight of the newborn was recorded with precision in each case preferably within one hour of delivery. Neonatal reflexes vix. feeding reflexes (Rooting, sucking, and swallowing), extensor reflexes (Moro's tonic neck reflex, Galants reflex, Perez reflex), progression reflexes (like stepping, placing reflex) were examined in each case and recorded on the predesigned proforma to correlate them with the gestational age of the baby.

Assessment of gestational age was done by using the physical and neurological characteristics laid down by Dubowitz et al (1970). Ten neurological characteristics were scored from 0-5, while eleven physical characteristics were scored from 0-4 in a predesigned proforma

and conversion of score into gestational age was done by the following formula or by the conversion curve (Dubowitz et al, 1970).

Estimated period of gestation = (R x 0.2642) + 24.5950
(in weeks)

Where 'R' represented the total score.

COLLECTION OF SAMPLE

Blood sample (10-12 ml) was collected from the cut end of umbilical cord from the placental side in the clean glass tubes, with due precautions to avoid contamination with maternal blood and haemolysis. All glassware used in the study were thoroughly sterilized and washed with distilled water and dried in hot air oven.

Blood samples were allowed to clot at room temperature. After 2-4 hours, serum was separated using a pippette and then serum was centrifuged at 1000 rmp for 15-20 minutes. After centrifugation 2 ml of clear serum at the top of sample was transferred to another dried vial with due marking on it. The samples were stored at +4°C and were analysed within 6-7 days for cholesterol and triglyceride.

METHOD FOR ESTIMATION OF CHOLESTEROL AND TRIGLYCERIDES

CHOLESTEROL

Total cholesterol was determined using the Henly's modification (1957) of Zlatkis, Zak & Boylos method (1953).

Principle

The red colour which cholesterol in acetic acid solution gives with ferric chloride and sulphuric acid is used as a measure of cholesterol concentration.

Reagents

i. Acetic acid (purified).

ii. Ferric chloride (0.05% solution).

iii. Sulphuric acid(concentrated).

iv. Stock cholesterol standard (100 mg/dl of purified acetic acid).

v. Working cholesterol standard - the stock cholesterol standard was dilured 1 to 25 with ferric chloride acetic acid.

Procedure

1. 0.1 ml of test serum was added to 6.5 ml of ferric chloride acetic acid reagent is a clean dry glass test tube marked 't' and mixed well.
2. The tube was allowed to stand for 20-25 minutes for proteins to flocoulate.
3. The contents of the tube 't' were centrifuged at 500 rpm for 15 minutes and then 5 ml of supernatent were transferred to another clean tube marked 't'.
4. For standard, 0.1 ml of working standard for use was added to 6.5 ml of ferric chloride in acetic acid solution and allowed to stand for 20-25 minutes. then contents were centrifuged at 500 rpm for

5 minutes and 5 ml of supernatent was transferred to another clean dry tube marked 'S'.

5. For blank, 5 ml of ferric chloride acetic acid reagent was taken in clean dry test tube marked 'B'.
6. How 3 ml of sulphuric acid was added to all three test tubes, 'T', 'S' and 'B' and mixed by inversion of stoppered tubes.
7. The tubes were allowed to stand for 30 minutes.
8. The standard 'S' and Test 'T' were read against blank 'B', using yellow filter or at 560 mn over a colorimeter.
9. The cholesterol (total) mg/dl was calculated by the formula :

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Reading of Test}}{\text{Reading of Standard}} \times 400$$

TRIGLYCERIDE

The triglycerides were determined by acetyl acetone method of Fletcher (1968) and Soloni (1971).

Principle

Triglycerides in serum were extracted with isopropanol. Triglyceride so estimated were saponified using alcoholic potassium hydroxide. Glycerol so released during saponification was oxidised with periodate to produce formaldehyde, which was reacted with acetyl acetone in presence of ammonium ions to produce yellow coloured diacetyl hydrolutidine, which

was measured colorimetrically.

1. Triglycerides + KOH - glycerol + Fatty acids.
2. Glycerol + periodate - Formaldehyde.
3. Formaldehyde + NH_4^+ + Acetyl Acetone - Diacetyl-lutidine (yellow).

Reagents

The reagents provided by Span Diagnostic Pvt. Ltd. were used for this estimation.

1. Haptane.
2. Isopropanol.
3. Sulphuric acid (0.08 N).
4. Potassium hydroxide 6.25 M
5. Periodate reagent.
6. Ammonium acetate.
7. Acetyle acetone.
8. Working triglyceride solution (200 mg/100 ml).
9. Working solution chromogen was prepared by mixing 0.1 ml of acetyle acetone and 14 ml of ammoxium acetate.

Procedure

A. Extraction of Triglyceride :

1. Three clean dry tubes were taken and each was labelled as 'B'(Blank), 'S'(Standard) and T(Test).
2. In blank'B' 0.5 ml of distilled water was taken and 2.0 ml of Haptane was added to it followed by 3.5 ml of Isopropanol and 1.0 ml of 0.08 N sulphuric acid. The contents were mixed well and allowed to

stand at room temperature for 10-15 minute till they separated in 2 layers.

3. In standard tube 'S' 0.5-1 of distilled water was taken. Then 0.5 ml of triglyceride standard (200 mg/dl) was added followed by 2.0 ml of Haptane, 3.0 ml of Isopropanol and 1.0 ml of 0.08 N sulphuric acid.
4. In the Test (T' tube, 0.5 ml separated serum was taken and to it 2.0 ml of Heptane was added followed by 3.5 ml of Isopropanol and 1.0 ml of sulphuric acid (0.08 N).
5. The contents of the tubes 'S' and 'T' were also mixed thoroughly and allowed to stand for 10-15 minutes till they separated into 2 layers.

B. Saponification and Colour Development :

1. Three clean dry tubes were labelled as 'B_1', 'S_1' and 'T_1' respectively.
2. Top solvent layer (0.4 ml) waa taken from tube 'B' and transferred to tube 'B_1'.
3. Similarly 0.4 ml of top solvent layer was taken from tube 'S' and 'T' and transferred to tubes 'S_1' and 'T_1' respectively using a clean glass pippette.
4. 2.0 ml of isopropanol and 1 drop of 6.25 M potassium hydroxide were added to each of three tubes 'B_1', 'S_1' and 'T_1'

5. The contents of tubes were mixed well and then the tubes were covered with alumina foil/cotton plub, and the tubes were kept in hot water bath (50-60°C) for 10 minutes and then cooled down to room temperature.

6. To these 0.2 ml of periodate reagent and 1.0 ml of chromogen (reagent 1) were added.

7. The contents of all three tubes blank 'B_1', Standard 'S_1' and Test 'T_1' were mixed well and the tubes were kept in hot water bath(50-60°C) for 10 minutes.

8. Then the tubes were cooled down to room temperature and the optical density of Standard (S_1), and Test (T_1) were determined against Blank (B_1) using a violet filter on a colorimeter.

C. Calculations :

Triglyceride concentration (mg/dl) :

$$= \frac{\text{Optical Density of Test (T)}}{\text{Optical Density of Standard (S)}} \times 200$$

Tabulation of Procedure for estimation of Triglycerides:

Sl. No.		Blank 'B' (ml)	Standard 'S' (ml)	Test 'T' (ml)
1.	Serum	-	-	0.5
2.	Distilled water	0.50	0.50	-
3.	Triglyceride standard	-	0.50	-
4.	Haptane	2.0	2.0	2.0
5.	Isopropanol	3.5	3.0	3.5
6.	Sulphuric acid	1.0	1.0	1.0
	Contents mixed well and allowed to stand at room temperature for 10-15 minutes, till they separated into 2 layers.			
7.	Top solvent layer	0.4	0.4	0.4
8.	Isopropanol	2.0	2.0	2.0
9.	Potassium hydroxide	1 drop	1 drop	1 drop
	Contents were mixed well. Tubes kept in hot water bath 50-60°C for 10 minutes. Cool down to room temperature.			
10.	Periodate reagent	0.2	0.2	0.2
11.	Chromogen	1	1	1
	Contents mixed well. Tubes kept in hot water bath (50-60°C) for 10 minutes. Cool to room temperature.			

Then optical density of standard and test determined against blank on colorimeter using a violet filter.

OBSERVATIONS

OBSERVATIONS

A study to determine the serum levels of cholesterol and triglycerides in cord blood of new born babies and their relationship to gestational age, birth weight and perinatal factors, was carried out on 51 newborn babies delivered at M.L.B. Medical College, Hospital, Jhansi between March, 1990 and November, 1990. Various clinical features were noted, birth weight was recorded and gestational age was assessed from first day of last menstrual period and was confirmed by the morphological and neurological criteria laid down by Dubowitz et al (1970).

A total of 51 cases were included in the present study. New born babies were classified according to maturity of child into preterm (<37 weeks), full term (37-41 weeks) and post term (>41 weeks) groups. There were 38(74.51%) cases in the full term group, 7(13.73%) cases in preterm group and 6(11.76%) cases in the post term group (Table 1).

TABLE - 1

Showing distribution of cases according to maturity by gestational age.

Sl. No.	Group	No.of cases.
1.	Pre term	7(13.73)
2.	Full term	38(74.51)
3.	Post term	6(11.76)
	Total	51(100.0)

Figures in parantheses indicate percentage)

The study group was also classified as per WHO criteria (1961) into two groups on the basis of birth weight. Newborns having birth weight less than 2500 gms were labelled as low birth weight (LBW) babies and this group included 12 (23.53%) cases, while newborns with birth weight of 2500 gms or more were designated as normal weight babies and this group included 39(76.47%) cases (Table 2).

TABLE 2

Showing distribution of cases on the basis of birth weight into normal and low birth weight groups.

Sl. No.	Groups	No. of cases
1.	Normal birth weight (birth weight 2500 gms and above)	39(76.47)
2.	Low birth weight (birth weight less than 2500 gms)	12(23.53)
	Total	51(100.0)

(Figures in parantheses indicate percentage).

An attempt was also made to classify the case material into appropriate for gestational age (AGA) and small for gestational age (SGA) groups according to the criteria laid down by Singh et al (1974). Full term group comprised of 37 AGA cases andl SGA case, preterm group included 4 AGA cases and 3 SGA cases. However in the post term group, all 6 cases were AGA. It is also evident from table 3 that maximum number of cases fell into the category of full term AGA group.

TABLE - 3

Showing distribution of study group into weight for gestational age groups.

Sl. No.	Groups		No.of cases
1.	Preterm	7	
	a. A.G.A.		4(57.14)
	b. S.G.A.		3(42.86)
2.	Full term	38	
	a. A.G.A.		37(97.37)
	b. S.G.A.		1(2.63)
3.	Post term	6	
	A.G.S.		6(100.0)
	Total	51	51

Further an attempt was also made to observe a correlation between the increasing gestational age to the values of cholesterol and triglycerides in this study. Accordingly, it was observed (Table 4) that 4(7.54%) cases were less than 33 weeks of gestation, 3(5.88%) cases were between 34-37 weeks, 27(52.74%) cases were 38-41 weeks, while 17(33.34%) cases were more than 41 weeks of gestation.

TABLE - 4

Showing distribution of cases according to increasing gestational age.

Gestational age(weeks)	No.of cases (%)
∠ 33	4(7.84)
34 - 37	3(5.88)
38 - 41	27(52.74)
7 41	17(33.34)

The sex of the baby was also given due consideration and accordingly there were 29(56.86%) male and 22(43.14%) female cases in the study group (Table 5).

TABLE - 5

Showing distribution of case material according to sex.

Sl. No.	Sex of baby	No.of cases	Percentage
1.	Male	29	56.86
2.	Female	22	43.14
	Total	51	100.00

TABLE - 6

Showing distribution of cases according to increasing birth weight.

Sl. No.	Birth weight (gm)	No.of cases	Percentage
1.	1000 - 1500	5	9.80
2.	1501 - 2000	3	5.88
3.	2001 - 2500	4	7.84
4.	2501 - 3000	35	68.63
5.	3001 & above	4	7.84
	Total	51	

Since no other workers have so far attempted to observe a correlation between increasing birth weight to the level of cholesterol and triglycerides in cord blood, it was tried to establish a correlation between them, if any, and accordingly the cases were distributed into various groups considering the increasing birth weight (Table 6). The distribution indicated 5(9.80%) cases in 1000-1500 gm range, 3(5.88%) cases in 1501-2000

gms range, 4(7.84%) cases in 2001-2500 gm range, 35(68.63%) cases in 2501-3000 gm range and 4(7.84%) cases in the group of birth weight more than 3001 gms.

It was also tried to observe and establish a correlation, if any between the cord cholesterol and triglyceride levels and the perinatal factors affecting the fetus. The various perinatal stress factors which were considered were (table 7) prolonged labour(7 18 hours), maternal hypertension and pre-eclamptic toxaemia, Antepartum haemorrhage, leading P/V 7 12 hours and birth asphyxia.

TABLE - 7

Showing the various perinatal stress factors affecting the fetus.

Sl. No.	Perinatal factors	No.of cases	Percentage
1.	Prolonged labour 7 18 hours	4/51	7.84
2.	Maternal hypertension and pre-eclamptic toxaemia	2/51	3.92
3.	Antepartum haemorrhage	3/51	5.88
4.	Leaking P/V 7 12 hours	8/51	15.68
5.	Birth asphyxia	5/51	9.80

It was seen that in few cases, more than one perinatal factors were involved and so the cases were also grouped according to the number of perinatal stress factors affecting the new born (Table 8).

TABLE - 8

Showing the number of perinatal factors affecting new born at a time.

Sl. No.	Group of factors	No.of cases	Percentage
1.	No perinatal stress factor	35	68.63
2.	1 Perinatal stress factors	11	21.57
3.	2 perinatal stress factors	3	5.88
4.	3 perinatal stress factors	2	3.92
	Total	51	

SERUM CHOLESTEROL AND TRIGLYCERIDE VALUES

All the results of serum cholesterol and triglyceride are expressed as mg/dl.

TABLE - 9

Showing mean ± S.D. values of cholesterol and triglyceride in various gestational age groups (mg/dl).

Groups		No.of cases	Cholesterol	Triglyceride
I.	Preterm	7	86.13±9.59	68.00±6.92
II.	Full term	38	70.50±12.73	37.33±10.09
III.	Post term	6	84.50±15.95	30.83±12.81

The mean±S.D. cholesterol values observed during this study were 74.22±14.26 mg/dl and triglycerides were 40.92±14.93 mg/dl. The mean cholesterol values ±S D observed for various maturity group i.e. preterm, full term, and post term were 86.14±9.59, 70.5±12.73 and 84.5±15.93 mg/dl respectively, while the values of

serum triglycerides in the above said groups were observed to be 68±6.92, 37.33±10.09 and 30.83±12.81 mg/dl respectively (Table 9).

It is clear from table 10 that the cord blood cholesterol values were lower in full term babies in comparison to pre term and post term groups, the difference being statistically significant($p < 0.01$ and < 0.05 respectively) (Table 10).

At the same time, the cord triglyceride values were lower in cases of full term and post term babies when compared to pre term babies and this difference was statistically highly significant ($p < 0.001$) (Table 10).

TABLE - 10

Showing statisticall analysis of cord cholesterol and triglyceride values as shown in table 9.

Compared groups of table 9	d.f.	Cholesterol 't'	Cholesterol 'p'	Triglyceride 't'	Triglyceride 'p'
I & II	43	3.099	< 0.01	7.679	< 0.001
II & III	42	2.424	< 0.05	1.415	> 0.05
I & III	11	0.227	> 0.05	6.657	< 0.001

d.f. = degree of freedom.

On comparison of the mean±S.D. values of cholesterol and triglycerides in the low birth weight (LBW) babies (birth weight $< 2,500$ gms) to normal weight babies (birth weight $> 2,500$ gms) (Table 11). It was observed that although cord blood cholesterol values were higher in the low birth weight babies group than their

normal weight counterparts (birth weight $\geq$2500 gm), the difference was statistically insignificant ($p > 0.05$). The triglyceride values were also higher in low birth weight babies than the normal weight babies, but unlike cholesterol, the difference in triglyceride values was found to be statistically highly significant ($p < 0.001$).

TABLE - 11

Showing serum values of cholesterol and triglycerides in low birth weight babies and normal weight babies(mg/dl).

Groups of babies	No.of cases	Cholesterol	Triglyceride
Normal weight babies (birth weight $\geq$2500 gm)	29	70.53±16.83	34.33±8.66
Low birth weight (LBW) babies(birth weight < 2500 gms).	12	78.20±13.06	62.33±10.19
't'		1.917	12.175
'p'		>0.05	<0.001
d.f. = 49			

TABLE - 12

Showing cholesterol and triglyceride values in various weight for age groups(mean±S.D. mg/dl).

	Groups	No.of cases	Cholesterol	Triglyceride
I.	Preterm			
	a. A.G.A.	4	89.75±8.13	67.00±4.35
	b. S.G.A.	3	80.00±8.16	69.33±8.21
II.	Full term			
	a. A.G.A.	37	70.44±12.70	36.75±9.13
	b. S.G.A.	1	80.00	66.00
III.	Post term:A.G.A.	6	84.50±15.93	30.83±12.81

It is evident from table 12 that the preterm group, the cholesterol values were higher in the AGA group than the SGA group, though no statistically significant difference was observed between the two($p > 0.05$).

TABLE - 13

Showing statistical analysis of various groups represented in table 12.

Compared groups	d.f.	Cholesterol 't'	Cholesterol 'p'	Triglyceride 't'	Triglyceride 'p'
Ia & Ib	5	1.567	>0.05	0.493	>0.05
Ia & IIa	39	2.630	<0.05	6.491	<0.001
Ia & III	8	0.601	>0.05	5.351	<0.001
Ib & IIa	38	1.274	>0.05	5.975	<0.001
Ib & III	7	0.439	>0.05	4.661	<0.01
IIa & III	41	2.432	<0.05	1.393	>0.05

In the full term group, since only one case was small for gestational age, no statistical significance could be observed in this group, though the only SGA case in this group had higher values of cholesterol than that observed in the AGA babies of this group. On statistical analysis of the cholesterol values in the appropriate for gestational age babies in preterm and full term group, it as observed that the former had much higher values than the latter, the difference being statistically significant ($p < 0.05$).

The serum triglyceride values were also seen to have a similar trend in the different weight for age

groups. As is evident from table 12 and 13, there was no statistically significant difference between the appropriate for gestational age and small for gestational age babies in the preterm ($p > 0.05$). In the full term group, though one small for gestational age baby demonstrated a higher value of triglycerides, no statistical significance could be derived. On comparison of triglyceride values in the pre term and full term appropriate for gestational age babies, the values were observed to be higher in the former than the latter, the difference being statistically highly significant ($p < 0.001$).

As has been detailed earlier, the cholesterol and triglyceride values observed with increasing gestational age (from ≤ 33 weeks to ≥ 41 weeks), to observe a correlation, if any between these parameters to the increasing gestational age. It is evident from table 14 that, in the premature groups (< 33 weeks and 34-37 weeks) statistically insignificant difference in cholesterol values was observed ($p > 0.05$). However, on comparison of the cholesterol values in both groups of preterm babies (< 33 weeks and 34-37 weeks of gestational age), to the values observed in the term (38-41 weeks) and more than 41 weeks gestational age group, a statistically significant difference was seen ($p < 0.001$ and < 0.05 respectively (Table 15).

These observations therefore confirm our earlier findings that the cholesterol values are higher and statistically significant in preterm group than the values observed in full term group.

Similar to the findings of cholesterol, the values of triglycerides had an inverse relationship with increasing gestational age. It was seen that amongst the preterm group (<33 weeks and 34-37 weeks) statistically significant difference was observed ($p > 0.05$), but statistically significant difference was observed when these values were compared with 38-41 weeks and more than 41 weeks gestational age group babies($p < 0.001$ in each case).

TABLE - 14

Showing cholesterol and triglycerides levels in increasing gestational age groups(Mean±S.D. mg/dl).

Gestational age (weeks)		No.of cases	Cholesterol	Triglyceride
I.	< 33	4	89.75±8.13	67.00±4.36
II.	34 - 37	3	80.00±8.16	69.33±8.22
III.	38 - 41	27	69.11±12.20	39.15±9.83
IV.	>41	17	77.64±15.19	32.38±10.76

In babies more than 41 weeks of gestational age the mean cholesterol values were higher and mean triglyceride values were lower in comparison to babies of 38-41 weeks of gestational age, these differences were statistically significant ($p < 0.05$).

TABLE - 15

Showing statistical analysis of observations of table 14.

Compared groups	d.f.	Cholesterol 't'	Cholesterol 'p'	Triglyceride 't'	Triglyceride 'p'
I & II	5	1.568	>0.05	0.492	>0.05
I & III	29	3.252	<0.001	5.523	<0.001
I & IV	19	4.816	<0.001	6.217	<0.001
II & III	28	7.276	<0.001	5.092	<0.001
II & IV	18	0.290	>0.05	16.675	<0.001
III & IV	42	2.653	<0.05	2.143	<0.05

TABLE 16

Showing mean ±S.D. values of cholesterol and triglyceride in different sexes (mg/dl).

Sex	No.of cases	Cholesterol	Triglyceride
Male	29	75.20±14.91	42.89±16.54
Female	22	73.28±12.97	36.09±12.25
	't'	0.481	1.143
	'p'	>0.05	>0.05
	d.f. = 49		

Table 16 denotes the mean ± S.D. values of cholesterol and triglycerides as observed in different sexes of this study group. Although males had slightly higher values of cholesterol and triglycerides (75.20± 14.91 mg/dl, 42.89±16.54 mg/dl respectively) than their female counterparts (73.28±12.97 and 36.09±12.25 mg/dl

for cholesterol and triglyceride respectively), these differences were statistically insignificant ($p > 0.05$).

TABLE - 17

Showing mean ± S.D. cholesterol and triglycerides in different birth weight groups (mg/dl).

Birth weight (gms)		No.of cases	Cholesterol	Triglyceride
I.	1000-1500	5	82.60±9.70	69.60±6.49
II.	1501-2000	3	88.86±6.18	63.5±3.57
III.	2001-2500	4	71.25±14.41	54.40±8.04
IV.	2501-3000	35	75.23±14.31	35.24±8.41
V.	3001 & above	4	61.25±5.07	22.83±5.17

TABLE - 18

Showing statistical analysis of observations of table 17.

Compared groups	No.of cases	Cholesterol		Triglyceride	
		't'	'p'	't'	'p'
I & II	6	0.955	>0.05	1.469	>0.05
I & III	7	0.535	>0.05	3.149	<0.05
I & IV	38	1.109	>0.05	8.734	<0.001
I & V	7	3.954	<0.01	11.698	<0.001
II & III	5	1.927	>0.05	1.799	>0.05
II & IV	36	1.596	>0.05	5.717	<0.001
II & V	5	9.138	<0.001	11.583	<0.001
III & IV	37	0.526	>0.05	4.332	<0.001
III & V	6	1.309	>0.05	6.605	<0.001
IV & V	37	1.920	>0.05	2.869	<0.01

An attempt was made to observe the mean cholesterol and triglyceride levels in different weight groups and to detect the relationship, if any between these values and increasing birth weight. Accordingly cases were classified into different weight groups and cholesterol and triglyceride levels were observed (Table 17) and statistically analysed (Table 18).

It is evident from table 17 that the cholesterol values were found to be higher in group I and II (1000-1500 and 1501-2000 gm respectively) and lowest in the group V viz one weighing 3001 gm and above.

On statistical analysis (Table 18) it was seen that only in these groups mentioned agove, a statistically significance was seen (I and V, $p < 0.001$, II and V, $p < 0.001$). However, in the III and IV group (birth weight 2001-2500 gm and 2501-3000 gms respectively), no statistically significant differences were observed either with group I and II and also with group V (3001 gm and above) ($p > 0.05$).

These findings highlight the fact that highest values of cholesterol are observed in the lowest birth weight group.

Contrary to the cholesterol values in the different birth weight group, on estimation of triglycerides, a decreasing trend was observed with increasing birth weight. A detailed statistical analysis amongst various groups is given in table 18.

It was seen that, although a highly statistically significant difference was seen in both group I and II to that of group V ($p < 0.001$), a significant difference was also seen on comparison of group IV to that of group V ($p < 0.01$).

During this study, an effort was also made to see the effects of various perinatal stress factors on the cholesterol and triglyceride contents of cord blood. We selected five main perinatal stress factors viz. Maternal hypertension, and pre-eclamptic toxaemia, prolonged labour (>18 hours), Ante partum haemorrhage, leaking P/V >12 hours and birth asphyxia and observed the mean cholesterol and triglyceride values in the affected group (Table 19 and 20). In all, 16 cases suffered from perinatal stress, in which few cases had simultaneously 2 or more factors involved (Table 21 and 22).

It was observed that the cases with history of leaking P/V more than 12 hours had higher mean $\pm$ S.D. cholesterol values (85.13 ± 11.57 mg/dl) in comparison to non affected individuals (71.74 ± 14.1 mg/dl), the difference being statistically highly significant ($p < 0.001$).

The mean$\pm$S.D. cholesterol values were lower in cases with history of Maternal Hypertension (67.0 ± 9.0 mg/dl) in comparison to non affected cases but the difference was statistically insignificant ($p > 0.05$).

The mean cholesterol levels were higher in cases with prolonged labour (77.2±9.6 mg/dl), antepartum haemorrhage (85.33±10.57 mg/dl and birth asphyxia (83.4±12.91 mg/dl) in comparison to non affected cases (71.74±14.1 mg/dl) but the difference was not statistically significant (p 70.05).

TABLE - 19

Showing various prenatal stress factors and cholesterol and triglyceride levels in these stress producing cases (Mean ± S.D. mg/dl).

Perinatal stress		No.of cases	Cholesterol	Triglyceride
I.	No perinatal stress	35	71.74±14.1	37.71±11.96
II.	Prolonged labour (718 hours).	4	77.20±9.6	38.25±3.24
III.	Maternal hypertension and preeclamptic toxaemia.	2	67.00±9.00	50.50±21.5
IV.	Antepartum haemorrhage	3	85.33±10.87	55.66±12.23
V.	Leaking P/V 712 hours	8	85.13±11.57	56.63±10.73
VI.	Birth Asphyxia	5	83.4±12.91	60.40±18.35

A very significant finding concerning the triglyceride values, was a significant correlation of the triglyceride values to most of the perinatal stress factors.

TABLE - 20

Showing statistical analysis of observations of table 19.

Compared groups	d.f.	Cholesterol 't'	Cholesterol 'p'	Triglyceride 't'	Triglyceride 'p'
I & II	37	0.750	>0.05	0.059	>0.05
I & III	35	0.466	>0.05	1.426	>0.05
I & IV	36	1.621	>0.05	2.492	<0.05
I & V	41	7.147	<0.001	4.106	<0.001
I & VI	35	1.745	>0.05	3.712	<0.001

The mean±S.D. triglyceride levels observed under different perinatal stress conditions showed that the triglyceride levels were markedly increased in cases with history of leaking P/V >12 hours (56.63±10.73 mg/dl) and birth asphyxia (60.4±15.35 mg/dl), in comparison to non affected individuals (37.71±11.96 mg/dl) and the differences were statistically highly significant($p < 0.001$). Raised triglyceride levels of statistical significance ($p < 0.05$) were also seen in cases with history of ante-partum haemorrhage (55.66±12.23 mg/dl) when compared to unaffected individuals ($p < 0.05$).

As few cases were affected with more than one perinatal stress producing factor at a time, so I tried to establish a correlation if any between the number of factors involved and the cholesterol and triglyceride values observed (Table 21). It was observed that the cholesterol values were slightly increased (79.45±13.12 mg/dl) in group with one perinatal stress factor in

comparison to that observed without any perinatal stress (71.74±14.1 mg/dl), but the difference was statistically insignificant ($p > 0.05$) (Table 22). Raised values of cholesterol were observed when 3 perinatal stress factors were involved simultaneously (93±1.0 mg/dl) in comparison to unaffected individuals, the difference was statistically significant.

It was observed that the mean±S.D. triglyceride levels exhibited an increasing trend as the number of perinatal stress factors involved increased.

TABLE - 21

Showing distribution of cases affected by perinatal stress producing factors and mean cholesterol and triglyceride values in these conditions (Mean±SD mg/dl).

Group	No.of perinatal stress producing factors	No.of cases	Cholesterol	Triglyceride
I	No	35	71.74±14.1	37.71±11.96
II	1	11	79.45±13.12	42.73±16.59
III	2	3	71.33±9.56	56.33±19.39
IV	3	3	93.0±1.0	64.0±4.0

TABLE - 22

Showing statistical analysis of observations in table 21.

Compared groups	d.f.	Cholesterl 't'	Cholesterl 'p'	Triglyceride 't'	Triglyceride 'p'
I & II	44	1.607	> 0.05	1.104	> 0.05
I & III	36	0.049	> 0.05	2.478	< 0.05
I & IV	35	2.104	< 0.05	3.063	< 0.01

The elevated triglyceride levels observed (42.73±16.59 mg/dl) when one perinatal factor was considered in contrast to levels observed in unaffected individuals (37.71±11.96) were not statistically significant ($p > 0.05$).

Elevated triglyceride values were also observed when 2 perinatal stress factors and 3 perinatal stress factors were considered (56.33± 19.39 mg/dl, 64±4 mg/dl respectively) in comparison to unaffected individuals. These differences observed were statistically significant($p < 0.05$ and < 0.01 respectively).

DISCUSSION

D I S C U S S I O N

The present work was carried out to study the serum levels of cholesterol and triglyceride in cord blood of 51 newborn babies. The study was conducted at M.L.B. Medical College, Jhansi, in the department of Pediatrics from March, 1990 to November, 1990. The primary aim of the study was to evaluate serum cholesterol and triglycerides in the preterm, full term and post term babies and to observe the correlation, if any, between their levels to the gestational age, birth weight and sex of the child. Further an attempt was made to observe the difference in the levels of cholesterol and triglyceride in babies affected by perinatal stress producing factors viz. maternal hypertension, prolonged labour, leaking P/V more than 12 hours, antepartum haemorrhage and birth asphyxia, and the babies unaffected by such perinatal stress producing conditions.

Besides evaluating serum cholesterol and triglycerides, weight was recorded and through physical examination of the newborn baby was done. The gestational age was calculated by counting the number of weeks from the first day of last menstrual period till the date of birth of child, and also by the scoring system using the morphological and neurological characteristics as suggested by Dubowitz et al (1970). Statistical analysis was done to derive mean$\pm$S.D. values of choles-

terol and triglycerides in various neonates, and the mean values were compared using the student's 't' test and the significance of the difference('p' values) was noted from the 't' distribution table. Based on observations depicted in various tables (Table 1-22). Various inferences have been drawn which are discussed herein detail.

A total of 51 cases were examined in this study The cases were grouped into preterm, full term and post term groups on the basis of gestational age. Considering the weight for age status as suggested by Singh et al (1974), the cases were further grouped as small for gestational age (SGA) and appropriate for gestational age(AGA) This study group comprised of 7 preterm(4 AGA and 3 SGA) 38 full term (37 AGA and 1 SGA) and 6 post term (All AGA) babies. Kumar et al (1989) had also grouped their cases into appropriate for gestational age and small for gestational age groups following the criteria laid down by Singh et al (1974). Sharma et al (1983) had classified their cases into small for gestational age group when their birth weight was below 10th percentile of Lubchenco's chart (1963). However, Haridas and Acharya (1984) and Mathur (1986) had classified their cases according to gestational age into pre term, full term and post term infants, without taking into consideration the weight for age status.

The birth weight alone, was also given importance and the study group was divided into low birth weight babies (⁄2500 gms) and normal weight babies (equal or more than 25,00 gms) as per definition of low birth weight babies given by WHO(1961). The low birth weight group included 12 babies(23.53%), while the normal weight group had a strength of 39(76.47%) babies. Mathur et al (1986) had also used the WHO definition (1961) for identification of low birth weight babies and observed cholesterol values in low birth weight and normal birth weight babies. Fosbrook and Wharton (1973) had also classified their cases into normal and low birth weight groups but they had used the criteria of birth weight less than 10th percentile for designating their cases as low birth weight ones.

The effect of increasing gestational age on cholesterol and triglyceride levels was also observed and accordingly the study group was divided into various groups in order of increasing gestational age, viz ⁄33 weeks, 34-37 weeks, 38-41 weeks and more than 41 weeks. Prakash et al (1989) had also studied lipid profile by classifying their study group on the basis of increasing gestational age.

The sex of child was given due consideration and accordingly the study group comprised of 29(56.86%) male and 22(43.14%) female cases.

An effort was made to observe and establish a

correlation if any, between the increasing birth weight and cholesterol and triglyceride levels, and so the case material was also grouped on this basis into 1000-1500 gm range, 1501-2000 gm range, 2001-2500 gm range 2501-3000 gm range and 3001 gms and above range. The maximum number of cases (68.63%) fell in the 2501-3000 gms birth weight group as indicated in table 6.

Most of the deliveries pass uneventfully and some of them are affected by various perinatal stress producing factors that exert their effect over the fetus in one way or the other. So, it was sought to establish a correlation if any, between these perinatal stress producing factors and cord cholesterol and triglyceride levels. In this study 16(31.37%) cases were affected by such factors while 35(38.63%) cases were not. The perinatal factors which were taken into consideration were prolonged labour, maternal hypertension, antepartum haemorrhage, leaking P/V more than 12 hours, and birth asphyxia. The effect of these factors over the above mentioned parameters were studied individually and collectively. Tsang et al (1974) had studied the effect of various parinatal factors on cord cholesterol and triglyceride levels individually and collectively, while Cress et al (1977) had selected hypercholesterolemic and hypertriglyceridemic babies after cord blood analysis and then studied the contribution of various perinatal stress producing factors retrospectively.

SERUM CHOLESTEROL AND TRIGLYCERIDES

The mean values ± S.D. of cholesterol and triglycerides, irrespective of birth weight, gestational age, sex and perinatal factors affecting the fetus were observed to be 74.22±14.26 mg/dl and 40.92±14.93 mg/dl respectively. The mean levels of cholesterol in cord blood had been estimated to be 69 mg/dl (Gyorgi et al, 1924), 67 mg/dl(Rafertedt et al, 1955), 82±17 mg/dl (Brown et al, 1959), 66 mg/dl(Brody and Carlson, 1962), 66 mg/dl (Kleeberg, 1963) and 79±21 mg/dl (Desai et al, 1977) in the past. The observations of this study have been in close proximity to those of above mentioned workers. However, Sadowsky (1947) Sohar et al (1956), Kaplan and Lee (1965) and Mathur et al (1986) observed higher values of cord blood cholesterol, as 107 mg/dl, 89 mg/dl, and 105.2±17.14 mg/dl respectively. Mean value of triglyceride levels observed in this study was 40.92± 14.93 mg/dl. These observations were in conformity of those by Brody and Carlson (1962) who recorded them to be 34 mg/dl and Mathur et al (1986) who observed them as 38±4.04 mg/dl. However, Desai et al (1977) had observed much higher values(62±21 mg/dl) than previous workers.

RELATION WITH MATURITY OF NEWBORN

Serum cholesterol and triglyceride values were observed in cord blood of 7 preterm,38 full term and 6 post term babies and as is evident from table 9, the

mean±S.D. values of serum cholesterol were 86.13±9.59 mg/dl in pre term, 70.5±12.73 mg/dl in full term and 84.5±15.95 mg/dl in post term babies. The cord cholesterol levels were raised in preterm and post term babies in comparison to full term babies with a statistically significant difference in each case ($p < 0.01$ and ≤ 0.05 respectively) (table 10). In the past Fosbrooke et al (1973) had observed no significant difference between the cholesterol levels in full term and pre term babies. Unlike the present study they observed higher values in full term as compared to pre term group. Similar to them, Mathur et al (1986) had also observed slightly higher levels in full term babies (112.2±14.58 mg/dl) Haridas et al (1984) had observed higher levels of cholesterol in cord blood of pre term babies (95±20.2 mg/dl) in comparison to full term babies (90±17.7 mg/dl), but contrary to this study the difference between the two values was statistically insignificant ($p > 0.05$).

Mean ±S.D. triglyceride levels observed in the present study were 68±6.92 mg/dl in preterm, 37.33± 10.07 mg/dl in full term and 30.83±12.81 mg/dl in post term babies. On statistical analysis the difference in triglyceride levels of pre term and full term and pre term and post term babies were highly significant ($p < 0.001$ in each case) while the difference between the levels observed in full term and post term was statistically insignificant ($p > 0.05$).

An interesting observation regarding the triglyceride levels in various maturity groups was that with the increment of maturity the triglyceride levels decreased, thus exhibiting an inverse relationship. These observations were in conformity to those by Haridas et al (1984) who obtained significantly higher levels of triglyceride in preterm babies (59±22.3 mg/dl) in comparison to full term (45±13.8 mg/dl), while Fosbrooke (1973) in contrast had obtained significantly lower levels of triglycerides in preterm babies (19.8±7.8 mg/dl) in comparison to full term babies (29.6±12.0 mg/dl)($p < 0.001$). The lower levels of triglycerides in preterm babies as observed by Fosbrooke (1973) have been attributed to lesser importance of fat metabolism in premature babies, while Haridas et al (1984) attributed the high level of triglyceride as observed in preterm, to stress full condition of preterm delivery.

No explanation as to the different values of cholesterol in preterm and term babies has been forth coming by any of the authors so far mentioned. The higher levels observed in this study, and by Haridas and Acharya (1984) in preterm babies could be attributed to more stress in the preterm babies than the term infants which is also the explanation given for the higher levels of triglyceride (both cholesterol and triglyceride being simple lipids) in the preterm group. The higher and significantly different levels of cholesterol in post term babies, when compared to term babies can be easily

explained due to its enhanced synthesis as a result of increase in enzymes and substrate necessary for its synthesis. This explanation has also been given by Haridas and Acharya (1984) who reported an increase in cholesterol during first few postnatal days after birth.

Regarding the raised levels of triglyceride in the preterm babies a possible explanation can be that preterm delivery is not a normal physiologic pehnomænon and it involves some amount of stress to fetus which may or may not manifest clinically (Kumar et al, 1989). Stress in any form has been shown to raise serum triglyceride level (Cress et al, 1977).

In this study the maturity was assessed by gestational age and an attempt was made to observe a correlation, if any, betweenthe increasing gestational age and levels of cholesterol and triglycerides in cord blood. It was observed that the levels of cholesterol in cord blood were higher (89.79±8.13 mg/dl) in the gestational age group ⁄33 weeks and they exhibited a downward trend as the gestational age advanced till term, the levels being 80±8.16 mg/dl, in 34-37 weeks gestational age group and 69.11±12.20 mg/dl in the 38-41 weeks gestational age group. In the group of gestational age 741 weeks, the cholesterol levels were observed to be increased (77.64±15.19 mg/dl). When the values observed in the gestational age group below 33 weeks and 34-37 weeks were compared to those observed in gestational age

group 38-41 weeks and more than 41 weeks, the differences were observed to be statistically significant ($p < 0.001$). Thus confirmed the earlier findings of this study, that cholesterol levels were raised in preterm group as compared to full term babies. Fosbrooke (1973), however, observed no significant change in cholesterol levels with increasing gestational age.

The triglyceride levels exhibited an inverse relationship with increasing gestational age (Table 14). The triglyceride levels were observed to decrease with increasing gestational age. When levels observed in gestational age groups below 33 weeks and 34-37 weeks were compared to the levels observed in 38-41 weeks and more than 41 weeks gestational age group, a statistically highly significant difference($p < 0.001$) was observed. These findings also supported earlier observations of significantly raised levels of triglycerides observed in preterm babies in comparison to full term and post term babies in this study. The raised levels of triglyceride in lower gestational age group had also been observed Haridas et al (1984). However, Fosbrooke et al (1973) observed an increasing trend in triglyceride levels along with advancement of gestational age, and they also observed that triglyceride levels did not increase much below 37 weeks of gestation, and beyond it the increase was significant in low birth weight groups.

RELATION WITH BIRTH WEIGHT

The relation of cord blood cholesterol and triglyceride levels with birth weight was studied by observing the variations in their levels in normal and low birth weight babies, in different weight for age groups and in different groups of increasing birth weight.

On comparing the above mentioned parameters in normal birth weight (equal or more than 2,500 gm birth weight) to low birth weight (less than 2,500 gms)babies (Table 11), the cholesterol levels were observed to be slightly raised in the latter group but the difference was insignificant ($p > 0.05$), while the triglyceride levels were observed to be increased significantly in latter group (62.33 ± 10.19 mg/dl) than the former one(34.33 ± 8.66 mg/dl)($p < 0.001$).

Fosbrooke and Wharton (1973) also had observed high levels of triglycerides in low birth weight babies (birth weight less than 10th percentile for age) in comparison to normal birth weight babies, the difference being statistically significant ($p < 0.05$), while they had observed no significant difference in cholesterol levels.

Mathur et al (1986) in contrast had observed statistically significant lower values of cholesterol in low birth weight babies (birth weight less than 2500 gm) in comparison to normal weight babies (equal to or more than 2,500 gm birth weight) who had higher levels. They

observed a positive correlation between cholesterol levels and birth weight.

A possible explanation for raised levels of triglycerides in low birth weight babies in comparison to normal weight babies can be, that the low birth weight group comprised mainly of preterm babies(appropriate for gestational age and small for gestational age) and full term small for gestational age ones. All these babies are supposed to facr intrauterine malnutrition and stress. As mentioned earlier stress in any form increases energy requirements(Kumar et al, 1989) for which the carbohydrate stores are inadequate and so fat mobilisation and catabolism takes over for energy requirements, there by increasing lipolysis which leads to raised serum triglyceride and free fatty acid levels. At the same time intrauterine malnutrition also favours adipose tissue breakdown liberating free fatty acids, and the portion of free fatty acids which excapes oxidation for energy is converted in the liver into triglycerides resulting in rise in blood triglyceride levels.

Due emphasis was also given to weight for age status and on observing the levels of cholesterol and triglycerides in different weight for age groups (Table 12 and 13), it was seen that in preterm group the cholesterol values did not differ much between the appropriate for gestational age (AGA) babies and small for gestational age (SGA) babies, though preterm AGA babies had an upper hand,

while in full term group the statistical significance of the difference between cholesterol levels in AGA and SGA could not be derived as there was only one case in SGA group though this solitary SGA case exhibited higher levels of cholesterol than AGA babies. On comparison of AGA babies of all 3 groups it was seen that preterm AGA babies and post term AGA babies had significantly higher cholesterol levels than full term AGA babies($p < 0.05$ in each case). This was in conformity to earlier observations and inferences that preterm babies had higher levels of cholesterol. These observations were in conformity to those by Kumar et al (1989) who also observed higher levels (100.09 ± 32.19 mg/dl) of cholesterol in preterm AGA babies in comparison to full term AGA babies (85.83 ± 22.85 mg/dl). They also observed higher values in preterm AGA than preterm SGA babies, the difference being statistically insignificant. However, the observations in this study differed from those of Kumar et al (1989) in the full term group, where they observed slightly higher values of cholesterol in AGA in comparison to SGA babies, whereas higher value (80 mg/dl) was observed in solitary SGA case than AGA babies (70.44 ± 12.70 mg/dl) in this study, the statistical significance could not be derived due to only a siggle SGA case.

The triglyceride levels in these groups of

weight for age also followed partly the pattern of cholesterol levels, with the levels being higher in the preterm groups in comparison to full term groups, the difference being statistically significant ($p < 0.001$). In the preterm group, the levels were raised in SGA babies in comparison to AGA ones. The difference was statistically insignificant ($p > 0.05$), while in the full term group, although the former had higher levels than the latter, statistical significance could not be inferred as the former group (SGA) consisted of only one case. These observations matched well with those of Kumar et al (1989) who had observed significantly raised triglyceride levels in preterm SGA babies in comparison to preterm AGA babies ($p < 0.01$), and also significantly raised levels in full term SGA babies in comparison to full termAGA babies.

The post term AGA babies exhibited no significance difference in cholesterol and triglyceride levels from the full term AGA babies.

The raised levels of triglyceride seen in SGA babies can be explained by considering them to be malnourished in intrauterine life, thereby switching over from carbohydrate metabolism to fat metabolism for energy requirements.

On observing the cholesterol and triglyceride levels in different groups of increasing gestational age (Table 17 and 18), high values of cholesterol were

noticed in lower birth weight groups (1000-1500 gm, and 1501-2000 gm birth weight group). Contrary to cholesterol levels, triglyceride levels demonstrated an inverse relation with increasing birth weights i.e. highest levels (69.6±6.49 mg/dl) in lowest birth weight group (1000-1500 gm) while lowest levels (22.8±5.17 mg/dl) in highest birth weight group (3001 cms and above). This observation of high levels of triglycerides in lower birth weight ranges can possibly be explained on the basis that these groups mainly comprised of preterm AGA and SGA babies, low birth weight babies (LBW) and full term SGA babies and as mentioned earlier, all these groups have demonstrated higher triglyceride levels, which have been explained by the stress of preterm delivery and intrauterine malnutrition.

RELATION WITH SEX

In the present study due consideration was given to the sex of the child and an effort was made to observe a difference, if any, between the two groups, regarding cholesterol and triglyceride levels. Although males exhibited slightly higher values of cholesterol (75.2±14.91 mg/dl) and triglycerides (42.89±16.54 mg/dl) than the females (73.28±12.97 mg/dl and 36.09±12.26 mg/dl respectively), but on statistical analysis these differences proved to be insignificant ($p > 0.05$ in each case). So it was inferred that the sex of the child

did not had any contribution towards changes in cord blood cholesterol and triglyceride levels.

RELATION TO PERINATAL STRESS

All deliveries are not uneventful and some cases are affected with perinatal stress. All factors affecting a neonate could not be studied. Five most important and commonly encountered ones were selected namely, maternal hypertension and pre-eclamptic toxaemia, prolonged labour, antepartum haemorrhage, leaking P/V $\geq$12 hours and birth asphyxia for this study.

On observing the cholesterol and triglyceride levels in cases affected by these factors individually (Table 19 and 20) it was noted that the levels of cholesterol increased with all these factors except maternal hypertension but these differences with unaffected cases were statistically insignificant except for the cases affected by leaking P/V $\geq$12 hours, where the difference was statistically significant ($p < 0.001$). Regarding triglyceride levels, a positive correlation with most of the perinatal stress producing factors was observed. The triglycerides levels were raised with all above mentioned factors (Table 19 and 20), but this rise was significant with antepartum haemorrhage ($p < 0.05$), leaking P/V more than 12 hours ($p < 0.001$) and birth asphyxia ($p < 0.001$), and insignificant with other ($p > 0.05$) when compared to unaffected cases.

On observing the cumulative effects of these perinatal stress factors on the cord cholesterol and triglyceride levels (Table 21 and 22) it was noticed that the cholesterol levels rose with increasing number of perinatal factors but the increase became significant with 3 perinatal factors affecting the fetus at a time ($p < 0.05$). The triglyceride levels exhibited a direct positive correlation with increasing number of perinatal stress factors and the difference was significant with 2 perinatal stress factors onwards ($p < 0.05$).

These findings were in close proximity to those of Tsang and Glueck (1974) who also observed significantly riased levels of triglycerides under stressful conditions individually and collectively($p < 0.01$ and < 0.005). They also observed that the triglycerides levels significantly increased with the increase in number of perinatal stress factors ($p < 0.005$). Cress et al (1977) had also observed the effects of perinatal stress over the biochemical parameters(cholesterol and triglycerides) in cord blood. They had selected hypercholesterolemic and hypertriglyceridemic neonates and analysed them for perinatal stress retrospectively. In their observations they recorded elevated cholesterol levels in prolonged labour, preterm, post term, birth asphyxia and leaking P/V more than 12 hours. High levels of triglycerides were observed in maternal hypertension,

prolonged labour, birth asphyxia. Tsang and Glueck(1974) and Cress et al (1977) had observed elevated triglyceride levels in increased perinatal stress. Lakhtakia et al (1990) had also observed raised levels of cholesterol and triglycerides in babies born to mothers with essential hypertension. The observations of raised triglyceride levels in babies born to mothers with hypertension during this study were in conformity to their recordings, however, on the contrary cholesterol values were not raised in the present study.

From the above said discussion it was inferred that triglyceride levels were more affected by perinatal stress in comparison to cholesterol levels. Under normal circumstances fetal energy requirements are nearly exclusively catered by oxidation of carbohydrate stores as the respiratory quotient at birth is nearly unity. Stress in utero, in birth canal leads to high energy requirements and thereafter depletion of glycogen and carbohydrate stores, so the energy requirements are then catered by fat mobilisation and utilisation along with increased synthesis of triglycerides in the liver. Also during stress sympathetic system is stimulated and catecholamines ellicit an immediate response on adipose tissue mobilisation and utilisation. All these mechanisms collectively lead to increased triglyceride levels at birth during stress.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was carried out to study the serum levels of cholesterol and triglycerides in cord blood of newborn babies. The cases were studied from March, 1990 to November, 1990, in the department of Paediatrics, M.L.B. Medical College, Jhansi(U.P.). The study group comprised of 51 newborn babies. The case material was divided into preterm, full term and post term groups according to gestational age of newborns. The babies were further classified into appropriate for gestational age(AGA) and small for gestational age(SGA) according to the intrauterine growth charts suggested by Singh et al (1974).

The primary aim of this study was to determine the level of serum cholesterol and triglycerides in newborn babies and to observe their correlation, if any, to the gestational age, birth weight and sex of the newborn babies. An attempt was made to compare the values of cholesterol and triglycerides in low birth weight group (birth weight less than 2,500 gms) to normal birth weight group (birth weight 2500 gms or more) and in babies affected by perinatal stress factors(maternal hypertension, prolonged labour, antepartum haemorrhage, leaking P/V more than 12 hours and birth asphyxia) to unaffected babies.

Besides evaluating serum cholesterol and triglyceride levels, thorough physical examination was done in each case. Gestational age of the baby was calculated by counting the number of weeks from the first day of last menstrual period till the birth of the baby and also by the criteria laid down by Dubowitz et al (1970). based on physical and neurological characteristics of newborn baby. Anthropometric measurements viz. head circumference chest circumferences, weight were recorded in each case. The perinatal stress factors affecting the individual cases were also noted.

SERUM CHOLESTEROL AND TRIGLYCERIDE IN NEWBORN BABIES

RELATION WITH MATURITY

Serum cholesterol and triglycerides were estimated in preterm, full term and post term babies. The observations revealed that the levels of cholesterol were raised in preterm and post term babies (86.13±9.59 mg/dl and 84.5±15.95 mg/dl respectively) in comparison to full term babies (70.5±12.73 mg/dl), the difference being statistically significant in each case ($p < 0.01$ and < 0.05 respectively). The triglyceride levels were observed to follow a downwards trend with advancement of maturity. The levels were 68±6.92 mg/dl in preterm, 37.73±10.09 mg/dl in full term and 30.83±12.81 mg/dl in post term. The differences between preterm and full term, and preterm and post term groups were statistically significant ($p < 0.001$ in each

case), while the levels in full term and post term babies did not differ significantly ($p > 0.05$).

The cholesterol and triglyceride levels were also observed in various groups in increasing order of gestational age. The cholesterol levels were observed to be higher in lower gestational age group (<33 weeks and 34-37 weeks) while these were lower in higher gestational age group (38-41 and >41 weeks). The triglyceride showed an inverse relationship with increasing gestational age.

It was concluded that triglyceride levels showed an inverse relations with gestational age.

RELATION WITH BIRTH WEIGHT

The relation with birth weight was studied by dividing cases into low birth weight (<2,500 gms) and normal weight groups (>2,500 gms), into appropriate for gestational age (AGA) and small for gestational age(SGA) groups , and into different groups on the basis of increasing birth weight.

On comparison of the above mentioned parameters in normal weight and low birth weight groups, the cholesterol levels were slightly increased in the latter groups. but not significantly, while triglycerides were significantly increased in low birth weight babies(62.33$\pm$10.19 mg/dl) in comparison to normal weight ones (34.33$\pm$8.66 mg/dl) ($p < 0.001$).

The cholesterol levels were raised in preterm AGA in comparison to preterm SGA, the difference being statistically insignificant ($p > 0.05$), while the levels in preterm AGA (89.75±8.13 mg/dl) and post term AGA (84.5±5.9 mg/dl) were higher than full term AGA with statistically significant difference ($p < 0.05$). The triglyceride levels were higher in preterm SGA in comparison to preterm AGA but the difference was statistically insignificant, while the preterm AGA babies exhibited significantly higher triglyceride levels (67±4.35 mg/dl) in comparison to full term AGA (36.75±9.13 mg/dl) and post term AGA babies (30.83±12.81 mg/dl) ($p < 0.001$ in both cases). Although one full term SGA baby had high value of triglycerides (66.0 mg/dl) in comparison to full term AGA (36.75±9.13 mg/dl) but no statistical significance could be derived owing to single full term SGA case.

On observing cholesterol and triglyceride levels in groups of increasing birth weight, it was observed that, cholesterol levels were towards higher side in lower birth weight groups (1000-1500 gm and 1501-2000 gm) in comparison to lower values in high birth weight group (3001 gm or more) the difference being insignificant. The triglyceride levels followed exactly inverse relation with increasing birth weight and accordingly were significantly higher (69.6±6.49 mg/dl) in lower birth weight group (1000-1500 gms) in comparison to low levels (22.83±5.17 mg/dl) in higher birth weight group (3001 gm or more) ($p < 0.001$).

RELATION WITH SEX

The present study group comprised of 29 males and 22 females. On statistical analysis of the values of cholesterol and triglycerides in male babies(cholesterol 75.2±14.91 mg/dl, triglyceride - 42.89±16.54 mg/dl) and in female babies (cholesterol- 73.28±12.97 mg/dl and triglycerides-36.99±12.25 mg/dl), it was found that the difference between the groups was insignificant($p > 0.05$) in both the groups.

RELATION WITH PERINATAL STRESS

During this study it was observed that triglyceride levels were more sensitive to perinatal stress and they were affected to a greater extent than cholesterol levels.

On analysing the effects of individual perinatal stress, leaking P/V >12 hours led to significantly raised levels of cholesterol (85.13±11.57 mg/dl, $p < 0.001$) in comparison to unaffected cases (71.74±14.1 mg/dl). In others the cholesterol levels were raised insignificantly ($p > 0.05$) in comparison to unaffected individuals except the newborn affected by maternal hypertension, whereas the cholesterol levels were insignificantly lower.

Triglyceride levels proved to be a much sensitive indicator of perinatal stress. The levels were raised insignificantly in cases with prolonged labour (38.25±3.24 mg/dl, $p > 0.05$) and maternal hypertension (50.5±21.5 mg/dl; $p > 0.05$), while the levels were

significantly increased in cases with antepartum haemorrhage (55.56±12.83 mg/dl, p $<$0.05), leaking P/V $>$12 hours (56.63±10.73 mg/dl, p $<$0.001) and birth asphyxia (60.4±18.35 mg/dl, p $<$0.001) in comparison to newborns unaffected by such factors (37.71±11.86 mg/dl).

On analysing the effects of various perinatal factors when they were acting simultaneously. It was seen that the cholesterol levels were significantly increased (93±1 mg/dl) when 3 or more perinatal stress factors were involved, in comparison to unaffected cases (71.74±14.1 mg/dl)(p $<$0.05), while the triglyceride levels were significantly raised (56.33±19.39 mg/dl and 64±4 mg/dl respectively) when two perinatal factors and three perinatal factors were involved simultaneously, in comparison to unaffected cases (37.71±11.96 mg/dl)(p $<$0.05 and $<$0.01 respectively).

The explanations which have been put forward to interpret the raised cholesterol and triglyceride levels in preterm babies in comparison to full term babies by other workers are also applicable here.

An additional feature of this study was that the mean values for post term babies have also been observed.

The observations of elevated triglyceride levels in cases of perinatal stress are in conformity to those by Western workers, and it is proposed that triglyceride levels are a more sensitive indicator for monitoring perinatal stress. However, study of free fatty acids and lipoproteins may throw more light on it.

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A P P E N D I X

WORKING PROFORMA

CORD BLOOD CHOLESTEROL AND TRIGLYCERIDE LEVELS IN NEWBORN BABIES

Case No.

MRD No. _____

Name : Sex : Male/Female

Date of birth :

Weight of birth

Mother's name Age :

Father's name Age :

Address :

Order of birth

Gravida Parity Abortion

ANTENATAL HISTORY

1. Fever.
2. Viral infection
3. Chronic Diseases : Respiratory
   Renal
   Cardiac
   Metabolic
   Any other
4. Antepartum haemorrhage
   (Bleeding in any Trimester)
5. Pre-eclamptic toxaemia
6. Maternal smoking
7. History of leaking P/V - ⁄12 hours, 7⁄12 hours.

NATAL HISTORY : Presentation
Mode of delivery - Normal vaginal
Forceps
Caessarean

Any feature of Birth Asphyxia

POST NATAL HISTORY

Birth Anoxia

Cyanosis

Delayed cry

EXAMINATION OF NEWBORN

I. Gen. Appearance : - Cry - Activity
- Colour - Posture

II. Anthropometric Exam.

Weight

Head circumference

Chest Circumference

III. General Exam.

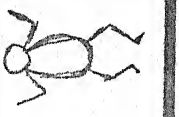
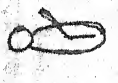
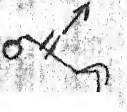
- Skin
- Head - A/F
- Face
- Oral cavity
- Neck and Trunk.
- Limbs
- Caput
- Cephalhaematonia
- Any other

IV. SYSTEMIC EXAMINATION

1. C.V.S.
2. Respiratory System
3. Abdomen
4. Neurological Exam.

Neonatal Reflexia

- Rooting
- Sucking
- Moros
- Reflex activity
- Any other.
- Galant reflex
- Perez reflex
- Stepping Reflex
- Placing Reflex

Neurological sign	SCORE					
	0	1	2	3	4	5
POSTURE						
SCARF WINDOW SIGN	90°	60°	45°	30°	0°	
ANKLE DORSI-FLEXION	 90°	 70°	 45°	 20°	 0°	
ARM RECOIL	 180°	 90-180°	 ∠90°			
LEG RECOIL	 180°	 90-180°	 ∠90°			
POPLITEAL ANGLE	 180°	 160°	 130°	 110°	 90°	 ∠9
HEEL TO EAR SIGN						
SCARF SIGN						
HEAD LAG						
VENTRAL SUSPEN-SION						

External Sign	0	1	2	3	4
Edema	Obvious edema of hands and feet; pitting over tibia	No obvious edema of hands & feet pitting over tibia			
Skin texture	Very thin gelatinous	Thin and smooth	Smooth: medium thickness, Rash or superficial peeling	Slight thickening Superficial cracking & peeling especially of hands & feet.	Thick and parchment-like, superficial or deep cracking
Skin colour	Dark red	Univormly pink	Pale pink; variable over body	Pale; only pink over ears, lips, palms, or soles.	
Skin opacity (trunk)	Numerous veins & venules clearly seen especially over abdomen.	Veins and tributaries seen	A few large vessels clearly seen over abdomen	A few large vessels seen indistinctly over abdomen.	No blood vessels seen
Lanugo (Over back)	No lanugo	Abundant; long & thick over whole back.	Hair thinning especially over lower back.	Small amount of lanugo and bald areas	At least 1/2 of back devoid of lanugo.
Plantar creases	No skin creases	Faint red marks over anterior half of sole	Definite red marks over 7anterior 1/2; indentation over 7anterior 1/3.	Indentations over 7anterior 1/3	Definite deep indentations over 7anterior 1/3
Breast size	No breast tissue palpable	Breast tissue on one or both sides 7 0.5 cm diameter.	Breast tissue both sides; one or both 0.5-1.8 cm.	Breast tissue both sides one or both 7 1 cm.	
Nipple formation	Nipple barely visible; no areola	Nipple well defined; areola smooth and flat, diam. 7 0.75cm.	Areola stippled, edge, not raised diameter, 7 0.75 cm	Areola stippled edge raised diam. 7 0.75 cm	
Ear form	Pinna flat&shapeless, little or no incurving of edge.	Incurving of part of edge of pinna.	Partial incurving whole of upper pinna.	Well-defined incurving whole of upper pinna	

External sign	0	1	2	3	4
Ear firmness	Pinna soft, easily folded, no recoil	Pinna soft, easily folded, slow recoil.	Cartilage to edge of pinna, but soft in places ready, recoil.	Pinna firm Cartilage to edge instant recoil.	
Genitals Male	Neither testis in scrotum	At least one testis high in scrotum	At least one testis right down.		
Female (with hips ½abducted)	Labia majora widely separated labia minora protruding.	Labia majora almost cover labia minora	Labia majora completely cover labia minora		

GESTATIONAL AGE (in weeks) = (Score x 0.2642) + 24.595

GESTATIONAL AGE : By L.M.P. =

By Scoring system assessment =

Sample collected on :

Investigation done on :

RESULTS : 1. Serum cholesterol -
2. Serum Triglyceride -
3. Weight -
4. Gestational age -
5. Weight for gestational age -
